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Biological Control

Biology and Biological Control of Knapweed

Linda M. Wilson and Carol Bell Randall

Forest Health Technology Enterprise Team

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Forest Service

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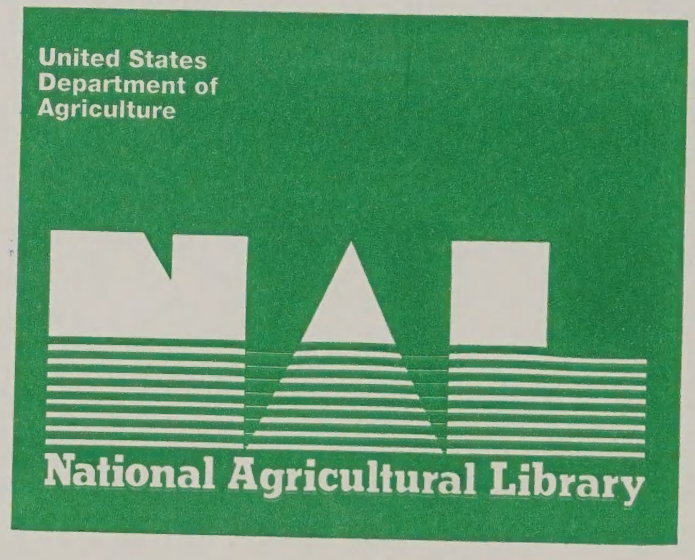

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Biology and Biological Control of Knapweed

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Introduction

Overview

The knapweeds comprise a diverse complex of species that predominantly infest rangelands in the western United States and Canada. This manual considers the biological control of six species of knapweeds: (1) spotted knapweed, (2) diffuse knapweed, (3) squarrose knapweed, (4) meadow knapweed, (5) black knapweed, and (6) brown knapweed. Spotted knapweed, *Centaurea maculosa*, is perhaps the most widespread species, followed in abundance by diffuse knapweed, *C. diffusa*. A third species, squarrose knapweed, *C. virgata* var. *squarrosa*, has a more limited distribution in the West. Three less widespread species, meadow (*C. pratensis*), brown (*C. jacea*) and black knapweed (*C. nigra*), are host to some of the same biocontrol agents and thus are included. Another well known species is Russian knapweed, *Acroptilon repens* (formerly known as *C. repens*). While a serious rangeland weed itself, Russian knapweed is not considered in this manual because it is sufficiently different from the other knapweeds to be considered separately and it has a unique complex of biocontrol agents.

In the United States, approximately 3.5 million acres are infested with knapweeds. A highly competitive and invasive groups of weeds, knapweeds have adapted to a wide range of habitats and environmental conditions. Although some of the most common rangeland pests in the West, knapweeds also invade pastures and fields in the Midwest and Eastern states. Spotted knapweed, for example, is widespread throughout the United States and found in all but four states (see Chapter 1 for maps of knapweed distribution in the United States).

A large amount of information is available giving the land manager good tools to manage knapweed by a variety of strategic methods. Chemical, cultural and mechanical methods used to control weeds all apply when managing knapweed. However, most people recognize that knapweed management on a large scale over the landscape requires a well-planned, integrated program that maximizes the effective use of all weed management strategies in combination.

Among the myriad of weed control approaches to manage knapweed is biological control, a well-known and long-established tool in the United States and Canada. Biocontrol of knapweed is one of the earliest, and diverse biocontrol of weeds programs in North America. There is a lot of readily available information describing knapweed biocontrol in general terms. Lacking, however, is a publication that describes knapweeds and their many biocontrol agents, combined with a how-to, on-the-job reference that outlines, step-by-step, the process of establishing a biocontrol program, including selecting a suitable site, collecting and releasing the biocontrol agents, and follow-up monitoring of the agents and the knapweed.

This manual provides a practical reference for field workers and resource managers when implementing a biological control program for knapweed. It includes information on selecting a release site, collecting and releasing new agents, evaluating past releases, redistributing established biocontrol agents, and monitoring agents and vegetation after the release. The guidelines and timetables outlined in this manual are based on research and practical field experience, and can be used to maximize the success of your knapweed biological control program.

Biological Control of Weeds

Biological control is the deliberate use of naturally occurring organisms to limit the distribution and abundance of a target weed. These are *natural enemies* of the weed in its native range (i.e.: Europe) and include such organisms as insects, mites, nematodes, and fungi. Natural enemies are also referred to as biocontrol agents, bioagents, biological control organisms and weed herbivores. Plant-eating insects and other organisms may control weeds by killing the weed directly, by weakening or stressing the weed, and by destroying seeds, root, or stems - thereby weakening the weed and limiting its reproduction. Secondary infection from pathogens that invade feeding lesions is indirect damage.

There are a number of advantages to biological control of weeds. Biocontrol with carefully selected agents is not damaging to the environment: it provides long-term impacts on the target plant; it has limited side effects; it is directed to a specific weed or closely related group of weeds; it has nonrecurring costs, and biocontrol agents are self-perpetuating.

Historically, biological control works best on large infestations of a single weed species. It has been most successful on weeds that have been introduced into areas where their specialized natural enemies do not occur. In the United States, knapweed, leafy spurge, rush skeletonweed, tansy ragwort, purple loosestrife, thistles and St. Johnswort are a few examples of weeds with established biocontrol programs.

Knapweeds were introduced from Europe without the complex of organisms that regulate their population densities. In a system known as *Classical Biological Control*, these natural enemies are identified in knapweed's native land, rigorously tested to determine what plants they eat (their *host range*), and finally imported and released into the environment.

It's very important that the candidate insect and weed are in synchrony. When initiating a biological control program, natural enemies are collected from areas where the weed is native. Specific areas are chosen that are climatically similar to the area where the weed is to be controlled. Ecological and genetic studies are needed to ensure that the lifecycle of the potential biocontrol agent is the same as the lifecycle of the target weed. Potential biocontrol agents undergo 5 to 10 years of rigorous testing to ensure that they eat only the target weeds and will in fact die without the weed. This is known as *host-specificity* and is the ecological cornerstone of biocontrol of weeds.

These preliminary studies are important in order to:

- Have the best fit between bioagents and knapweed
- Preclude introduction of unapproved organisms
- Protect nontarget species, such as crop plants or rare and endangered plants
- Influence future assessments of risk
- Affect future evaluation processes

The USDA Animal and Plant Health Inspection Service (APHIS) is the governing agency responsible for authorizing the importation of an insect and other organisms for biological control of weeds. Rigorous laws and regulations are in place to minimize risks associated with introducing foreign organisms. Biocontrol researchers work closely with APHIS to maximize safety in biocontrol programs.

While biocontrol claims to be an effective and important weed management tool, it is not a panacea; it does not 'fix' the problem of knapweed. In the most effective programs, biological control is used along with other methods of weed control. In fact, many land managers, ranchers, and farmers use integrated weed management systems, which combine more than one method to manage weeds while keeping the ecosystem intact. The article listed in the Selected References section entitled, *Recent Developments in Biological Control of Weeds*, provides a review, examples, and a discussion about the advantages and disadvantages of different approaches used to control weeds using biological methods.

About This Manual

This manual provides background information on each of the six knapweed species listed above, detailed descriptions of 13 knapweed biocontrol insects, and elements of a knapweed biocontrol program. The chapters are:

Chapter 1 provides detailed discussions of each of the knapweed species included in this manual. The species are identified by their scientific name, description of the leaves, stems, flowers, seeds, and habitat and occurrence in the United States. Photographs, drawings, and distribution maps are also provided.

Chapter 2 features knapweed biocontrol agents (flies, moths, and beetles) and their basic biology, including information on identification and lifecycle of each of the knapweed biocontrol agents. Information in this chapter is particularly useful in being able to identify each biocontrol agent in the field. Eight species of seedhead feeders and five root borers are described.

Chapter 3 includes detailed elements of a knapweed biocontrol program (planning, implementing, and evaluating). It encompasses techniques for all the agents. Included are guidelines for:

- Developing work schedules for field activities
- Selecting and preparing a release or nursery site
- Collecting, handling release, transporting and shipping biocontrol agents
- Monitoring agents and vegetation at the release site

Glossary defines technical terms essential in using and communicating about biological control effectively.

Selected References covers critical references from the comprehensive body of literature on knapweed biology, ecology, and biological control.

Appendices A-G contains various insect release and monitoring forms, checklists, vegetation monitoring forms, and most important, a troubleshooting guide.

Appendix A: Troubleshooting Guide: When Things Go Wrong

Appendix B. Sample Biocontrol Agent Release Form

Appendix C: Monitoring Plan Checklist

Appendix D: Biocontrol Monitoring Report

Appendix E: Qualitative Monitoring Form

Appendix F: Quadrat Density and Cover Data Form

Appendix G: Macroplot Design for Measuring Density

Chapter 1:
Getting to Know Knapweeds

Knapweeds belong to the genus *Centaurea* and are members of the Sunflower family (Asteraceae). This is a very large and diverse family of plants that includes dandelions, sunflowers, and daisies. Most knapweeds are non-native to North America. They originated from Europe and Asia and were brought to North America following the immigrant trail from those regions. Together, these Eurasian knapweeds form a large complex of invasive species that are found throughout the United States and Canada. All told, 25 species of knapweeds occur in the two countries, predominantly as noxious rangeland weeds in the West. Six species are considered in this manual. Among the most troublesome are diffuse, spotted, and squarrose knapweeds. Lesser-known knapweeds (meadow, brown, and black) are closely related to the others and are included in this manual because they share similar biology and some of the same biological control agents.

Knapweeds are highly invasive weeds that are capable of large infestations under favorable conditions (Fig. 1). Knapweeds are distinguished by their bract shape, flower color, leaf shape, roots, seeds and branching habit. The taxonomic key (*Key to the Knapweed Species, p. 7*) can be used to identify the six knapweed species described in this manual. Sections following the key describe each of the species separately in greater detail to enable the user to identify each species in the field.

Figure 1. Spotted knapweed infestation in northern Idaho.

The list of references (*Selected References*, page 81) provides additional information about knapweed species discussed here.

Plant Development

All six knapweed species begin their lifecycle as seedlings that develop into prostrate rosettes of 5 to 12 lobed leaves (Fig. 2). Most species remain rosettes the first year. With the onset of warm, moist conditions the following spring, plants bloom on one to several branched, flowering stems. Plants have many seedheads that occur singly at the tips of branched stems.

Figure 2. Spotted knapweed rosette.

Like all members of the sunflower family, the knapweed seedhead, or *capitulum*, is an aggregation of 20-40 small, individual flowers (Fig 3a). The individual flowers, or florets, are tightly clustered and anchored to a concave base, called the *receptacle*. The receptacle and florets are surrounded by an envelope of modified leaves, or *bracts*. Flower color and bracts are important diagnostic characters for knapweeds.

As the seedhead completes its development, the bracts separate to reveal the florets, enabling pollination to occur. Seeds develop later in the season (knapweed seeds are also known as *achenes*). Seeds are generally, but not always, plumed with white bristles.

Insects used in knapweed biological control inflict damage to the plant in two places: the seedhead and the root. The plant is damaged by the larvae of these insects which feed on the seedhead or root tissue and destroy plant tissue. Only the adult seedhead weevils eat foliage, otherwise adult insects generally don't damage the plant.

Seed-feeding biocontrol agents attack the plant at specific stages of seedhead development; thus some insects attack the plant early in development, when in the bud stage, and some that attack later in development when plants are in early to full bloom. Larvae eat and destroy seeds.

Root-boring biocontrol agents can attack the plant as soon as the root is large enough for the insect to feed. The root is composed of two key tissues: the root cortex and the central vascular tissue (Fig. 3b). Both tissues are nutritious; the cortex tissue is used to store nutrients and the vascular tissue contains the channels in which nutrients and water move up and down the plant.

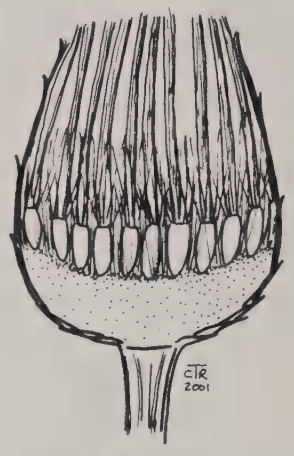


Figure 3a. Knapweed capitulum showing placement of florets.

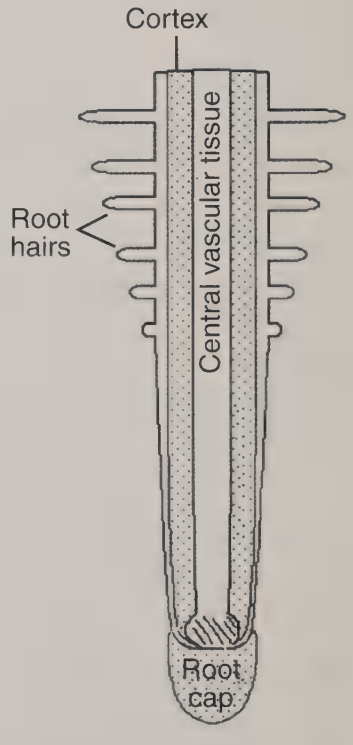


Figure 3b. Key tissues in the knapweed root.

Key to the Knapweed Species

(Adapted from Roché and Roché 1993)

A1. Bracts that surround the flower head are spine-tipped, biennial or short-lived perennial

B1. Central, terminal bract bent backwards (recurved)

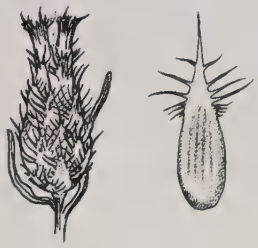
Squarrose knapweed

C. virgata ssp. *squarrosa*

B2. Central, terminal bract, spreading (not recurved)

Diffuse knapweed

C. diffusa



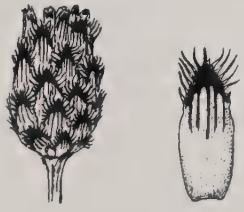
A2. Flower heads without spine-tipped bracts

C1. Edge of bract is a comb-like fringe

D1. Fringes of bracts short, drawn out and rigid, bract with brown triangular tip

Spotted knapweed

C. maculosa



D2. Fringes on bracts as long or longer than the width of the bract, not rigid

E1. Fringe on bract black

Black knapweed

C. nigra

E2. Fringe on bract tan to brown

Meadow knapweed

C. pratensis

C2. Bracts without comblike fringe, having a brown, papery, translucent tip

Brown knapweed

C. jacea

Spotted Knapweed

Scientific name: ***Centaurea maculosa* Lam.**

A winter-hardy, short-lived perennial with long, fibrous taproots. Plants (Fig. 4) grow 6 to 24 inches (15 to 60 cm) in height and spread entirely by seeds. It is native to eastern Europe and Asia.

Leaves: The basal rosette leaves are up to 8 inches (20 cm) long, deeply lobed, and arranged in a rosette. Stem leaves, arranged alternately along the stem, are smaller and not lobed. Uppermost leaves are bract-like.

Stems: The stems are upright, stiff, and branched. Small plants usually have an unbranched stem and one flower head; large plants have a stem with many branches and can have over 100 flower heads.

Flowers: Flowering occurs from June to October. The 0.2 to 0.4 inch (5-10 mm) long flower heads occur singly or in clusters at the branch tips. Each head bears stiff bracts; the terminal bract is black-tipped giving the plant its 'spotted' appearance. Heads contain from 30 – 50 pink or purple colored flowers (Fig. 5).

Seeds: Seeds are 0.1 inch (2.5 mm) long, oval, black or brown with pale, vertical lines. Each seed has a short, bristly pappus of bristles about half the length as the seed (Fig. 6). Each plant can produce up to 600 seeds that can remain dormant for many years.

Habitat and Occurrence: Spotted knapweed grows in a wide range of habitats, though mainly in grasslands and open forests. It has the widest distribution in the United States of all the knapweed species (Fig. 7). A rapid colonizer of disturbed land, spotted knapweed can displace native vegetation in undisturbed areas. Flower heads persist on the stiff stems through the winter eventually breaking off when new rosette growth appears the following spring.

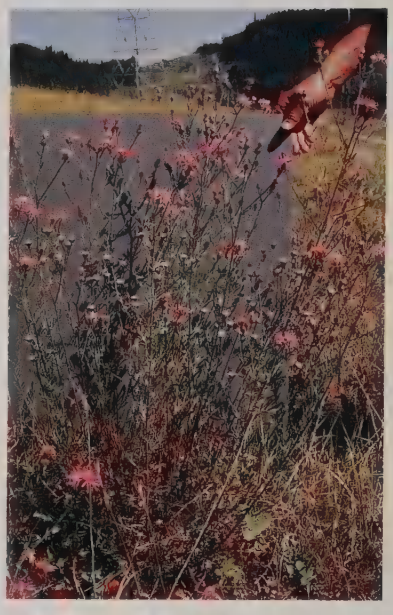


Figure 4. Spotted knapweed plant.

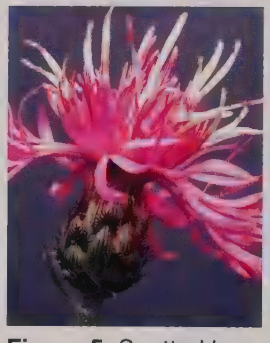


Figure 5. Spotted knapweed seedhead.

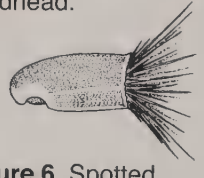


Figure 6. Spotted knapweed seed.

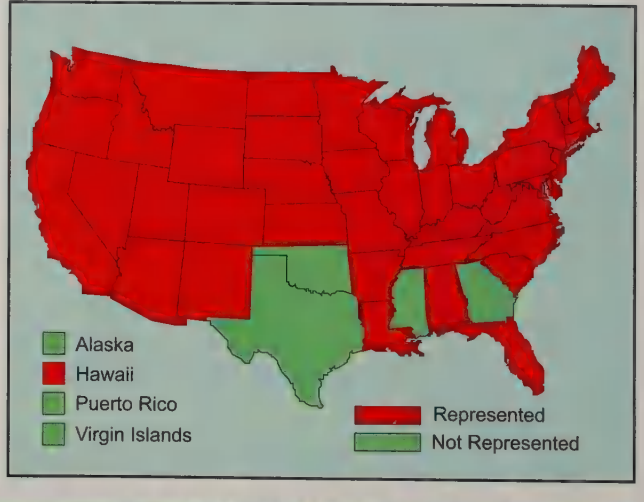


Figure 7. Map of spotted knapweed distribution in the United States.

Diffuse Knapweed

Scientific name: ***Centaurea diffusa* Lam.**

A winter-hardy biennial or short-lived perennial that reproduces entirely by seeds. Diffuse knapweed (Fig. 8a) is originally from the eastern Mediterranean.

Leaves: The deeply lobed basal leaves are up to 4 inches (10 cm) long and 1 inch (2.5 cm) wide and arranged in a low-lying rosette. Lower stem leaves are alternate and divided into many lobes, whereas upper stem leaves are much smaller and have only a few slender lobes.

Stems: The single upright stem grows 6 to 24 inches (15 to 60 cm) in height and has numerous branches mostly on the upper half.

Flowers: Flowers are predominantly white, occasionally pink-purple (Fig. 8b). Heads are 0.5 inch (1.3 cm) long and covered with small, narrow bracts ending in sharp, rigid spines. The terminal spine is distinctively longer and spreading. Flowering occurs from June to October.

Seeds: Seeds are 1/8 inch (5 mm) long, oblong, and dark brown. Rarely will seeds have a pappus of short, pale bristles (Fig. 8).

Habitat and Occurrence: Diffuse knapweed is adapted to a wide range of habitats. Although it prefers dry, bunchgrass zones of the Intermountain West, it is also found in the Midwest and the East (Fig. 9).

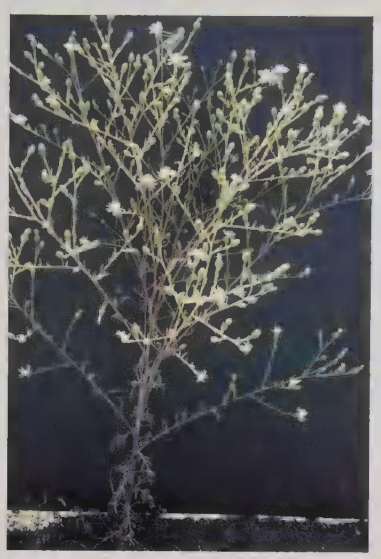


Figure 8a. Diffuse knapweed plant.

Figure 8b. Diffuse knapweed seedhead.

Comments: Like spotted knapweed, diffuse knapweed can displace native vegetation in undisturbed areas. Specialized chemicals give this weed a distinctive smell and an extremely bitter taste. Unlike other knapweeds, the heads of diffuse do not open to shed seeds. Instead, seeds are shed when the stiff, mature plants break off and tumble in the wind. Seeds are also spread by vehicles, animals, and people.

Figure 9. Diffuse knapweed seed.

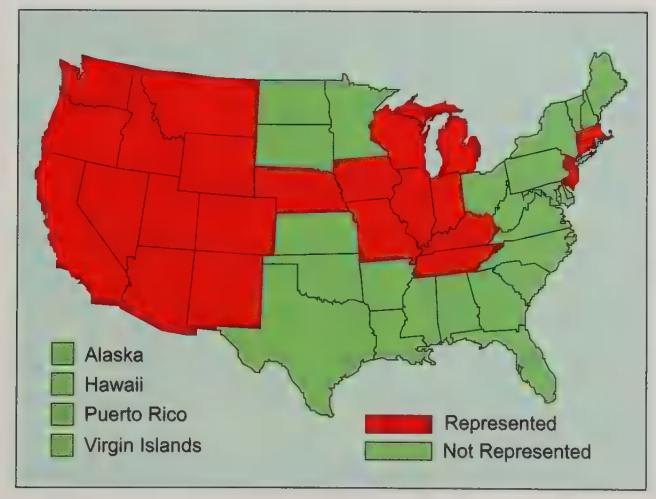


Figure 10. Map of diffuse knapweed distribution in the United States.

Squarrose Knapweed

Scientific name: ***Centaurea virgata* Lam ssp. *squarrosa* Gigl.**

Squarrose knapweed (Fig. 11a) is a long-lived perennial with deep tap roots that reproduces only by seed. Squarrose knapweed came to the United States in 1876 from the eastern Mediterranean.

Figure 11a. Squarrose knapweed plant.

Leaves: Rosettes of deeply lobed, gray-green leaves characterize squarrose knapweed.

Stems: The stems are upright, stiff, winged and branched. Small plants usually have an unbranched stem and one flower head; large plants have a stem with many branches and can have over 100 flower heads. Plants range in height from 6 to 24 inches (15 to 60 cm).

Flowers: Flowering occurs from July to September. Flower heads with 4-8 pink or purple flowers are borne singly or in pairs at the tips of branches. The seedheads (Fig. 11b) are small and covered with spiny bracts having a long, recurved (backward pointing) terminal spine. The heads are deciduous, falling off the stems soon after the seeds mature.

Figure 11b. Squarrose knapweed seedhead.

Seeds: Squarrose knapweed seeds (Fig. 12) are pale to dark brown with pale vertical stripes and a short, white pappus. Only 3-4 seeds are produced per head, each measuring about 1/8 inch (5 mm) in length. Seeds are dispersed individually as they fall from the heads and collectively when whole plants break off and tumble in the wind. Seeds are also dispersed when whole heads break off from the stem and get lodged in the hair and fur of animals, much like cockleburs and burdock.

Figure 12. Squarrose knapweed seed.

Habitat and Occurrence: Squarrose knapweed has a limited distribution in Utah, Oregon, California and Wyoming and Michigan (Fig. 13). It prefers dry, open rangeland with shallow soils.

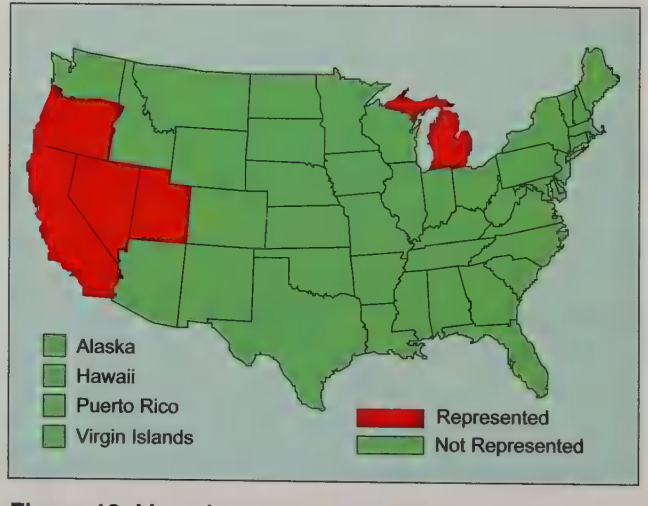


Figure 13. Map of squarrose knapweed distribution in the United States.

Comments: Squarrose is similar to diffuse knapweed but has the recurved spines on the bracts and is a true perennial.

Meadow Knapweed

Scientific name: ***Centaurea pratensis* Thuill.**

This native of central Europe is a deep-rooted, bushy perennial, growing each year from a woody root crown.

Leaves: Basal leaves are up to 6 inches (15.2 cm) long, tapering at both ends and having the broadest part above the middle of the leaf. Stem leaves are lance-shaped, shallowly-lobed and stalkless.

Stems: There are usually few to several stems with many branches. Mature plants reach 3.5 feet (1.04 m) tall (Fig. 14a).

Flowers: Flowers are generally rose-purple in color, although white flowers occasionally occur. Flowering occurs from July to September. The heads are solitary at the ends of the upper branches. They are broadly oval and almost globe-shaped, 0.5 inch (1.3 cm) long. The bracts of meadow knapweed are light to dark brown, with a papery fringe on the margin (Fig. 14b).

Seeds: Meadow knapweed seeds are pale tan in color, plumeless, 1/8-inch (2 cm) long (Fig. 15).

Habitat and Occurrence: Meadow knapweed prefers moister and cooler conditions than the other knapweeds. It occurs predominantly in coastal Washington and Oregon, but is also found in cooler regions of the interior (Fig. 16).

Comments: Meadow knapweed is a fertile hybrid between black and brown knapweeds.

Figure 14a. Meadow knapweed plant.

Figure 14b. Meadow knapweed seedhead.

Figure 15. Meadow knapweed seed.

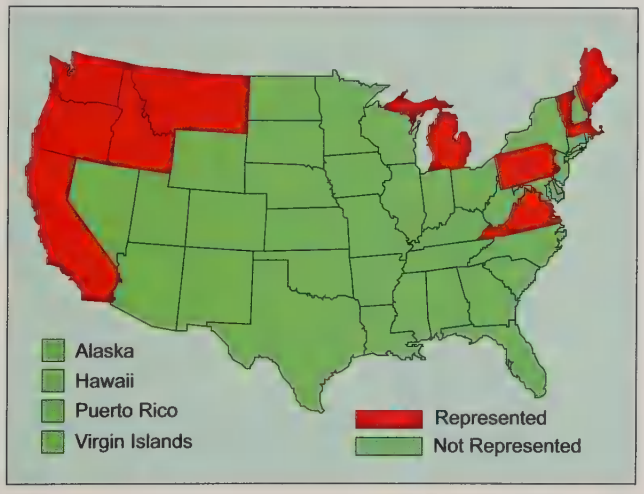


Figure 16. Map of meadow knapweed distribution in the United States.

Black Knapweed

Scientific name: ***Centaurea nigra* L.**

Black knapweed is a perennial plant regrowing each year from a woody root crown (Fig. 17). It was introduced into the United States in 1876 from the United Kingdom.

Leaves: Basal rosette leaves are broad, stalked, and shallowly lobed. Stem leaves are smaller and not lobed.

Stems: Stems are erect and branched near the middle, from 8-32 inches (20 to 80 cm) tall, the base of the stem is sometimes prostrate and rooting from the nodes.

Flowers: Flowering occurs from July to October. Flowers are rose to lavender colored. Heads occur solitary at the ends of the upper branches. They are broad and rounded, 0.5 inch (1.3 cm) tall and 1 inch (2.54 cm) wide. The bracts of black knapweed are black, with a comb-like fringe on the margin.

Seeds: Black knapweed produces about 60 seeds per head. They are ivory with lengthwise stripes, with a pale and short plume (Fig. 19).

Habitat and Occurrence: Like meadow knapweed, black knapweed occurs predominantly in coastal Washington and Oregon, in cooler regions of the inland Northwest (Fig. 20).

Figure 17. Black knapweed plant.

Figure 18. Black knapweed seedhead.

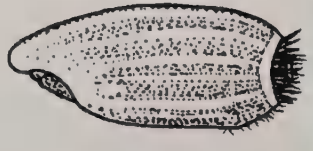


Figure 19. Black knapweed seed.

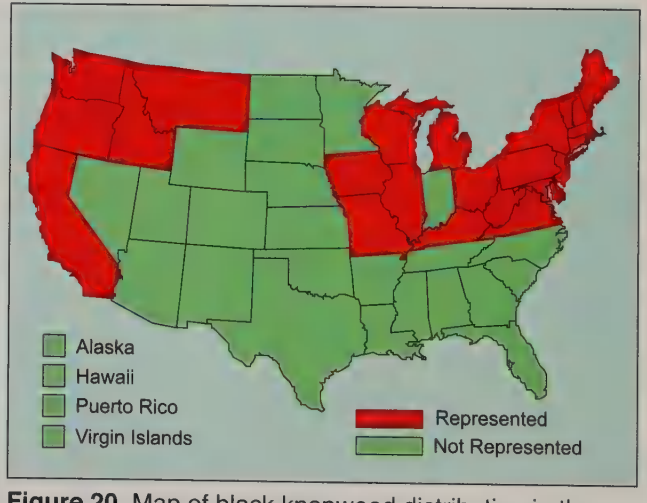


Figure 20. Map of black knapweed distribution in the United States.

Brown Knapweed

Scientific name: ***Centaurea jacea* L.**

Brown knapweed (Fig. 21a) is a perennial that reproduces only by seeds. It is native to Europe.

Leaves: Basal leaves are up to 6 inches (15.2 cm) long, tapering at both ends with the broadest part above the middle of the leaf. Stem leaves are lance-shaped, shallowly-lobed and stalkless.

Flowers: Flowers are rose-purple in color, occasionally white flowers occur. Flowering occurs from July to October. Heads are solitary at the ends of the upper branches (Fig. 21b). They are broadly oval. The bracts of brown knapweed are light to dark brown, with a papery fringe on the margin.

Seeds: Brown knapweed seeds (Fig. 22) are pale tan in color, plumeless, 1/8 inch (2 cm) long. Each head produces about 12 seeds.

Habitat and Occurrence: Like meadow knapweed, brown knapweed prefers moister, cooler conditions than the other knapweed species. It occurs predominantly in coastal Washington and Oregon, although it is distributed both in the West and the East (Fig. 23). It also occurs in British Columbia.

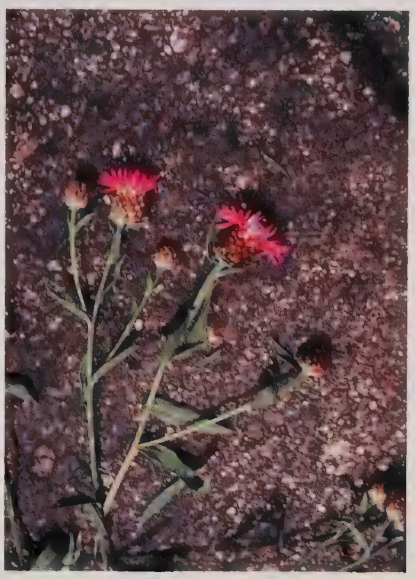


Figure 21a. Brown knapweed plant.

Figure 21b. Brown knapweed seedhead.

Figure 22. Brown knapweed seed.

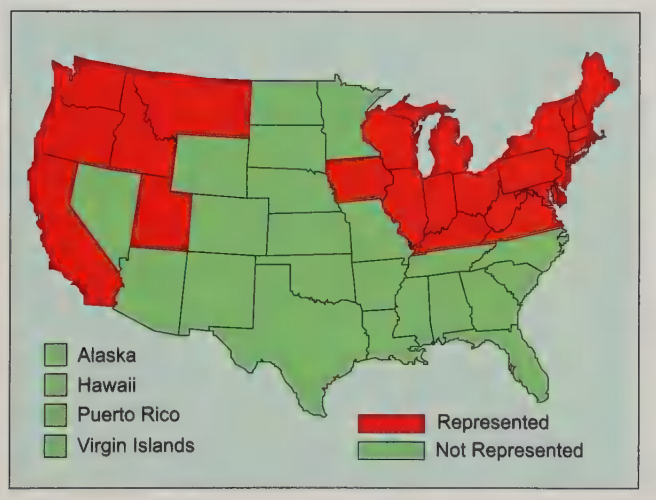


Figure 23. Map of brown knapweed distribution in the United States.

Chapter 2:
Biology of Knapweed Biocontrol Agents

Biological control of knapweeds is one of the oldest classical biocontrol programs in the United States and Canada. It began in the 1960's with the importation of two seedhead flies: the knapweed banded gall fly, *Urophora affinis*, and the UV knapweed seedhead fly, *U. quadrifasciata*. In all, 16 agents have been studied; of these, 13 are insect species, 2 are fungi, and 1 is a mite (not released). Only the insects are emphasized in this manual because they are by far the most widespread, readily available and easy to work with. This chapter is organized into two sections: seedhead-feeding (seedhead feeders) and root-boring (root borers) insects.

Basic Insect Biology

Insects are a diverse and complicated group of animals. Basic knowledge of insect anatomy and lifecycles will help a great deal in recognizing knapweed bioagents in the field and understanding their impact on the weed. Adult insects possess unique characteristics: (1) an exoskeleton, (2) a segmented body consisting of three regions: head, thorax and abdomen, and (3) three pairs of legs (Fig. 24).

Insects grow and develop through a series of stages. The transformation from egg through juvenile stages to adult is called *metamorphosis*. This process can be incomplete or complete. All the insects used in biocontrol of knapweed undergo complete metamorphosis (having four distinct life stages): egg, larva (of which there can be three or more *instars*), pupa, and finally, adult.

The insect bioagent's lifecycle (Fig. 25) is closely matched, or *synchronized*, with knapweeds. In fact, in order to qualify as an acceptable biological control agent, the insect must show that it eats and develops only on knapweed and no other plants. Without knapweed, a specific complex of knapweed, the insect will die. This highly specific, tightly regulated insect-plant relationship is the most critical issue in classical biological control of knapweeds.

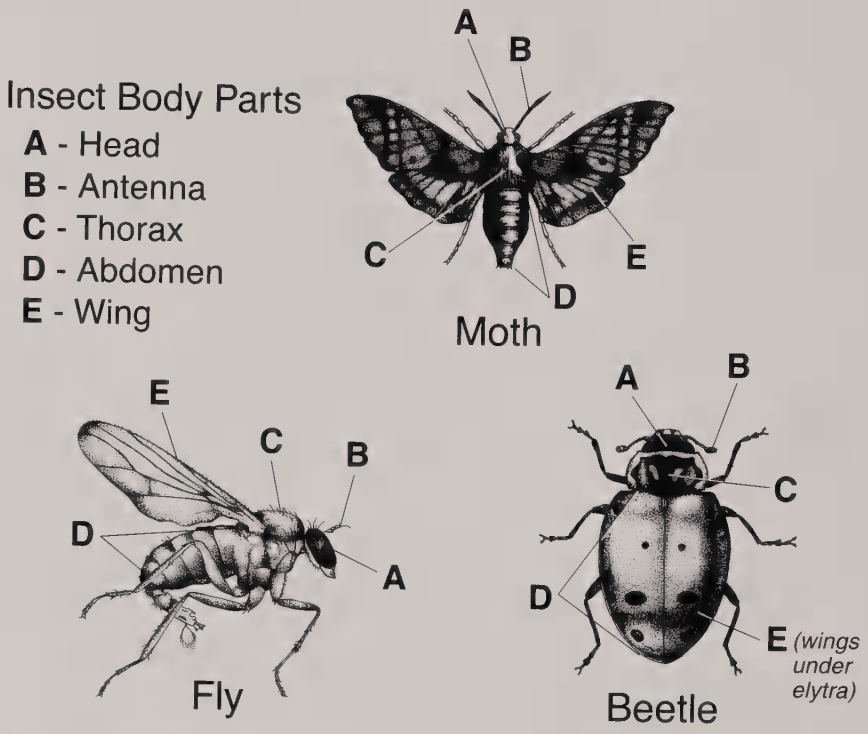


Figure 24. Diagram of insect body parts.

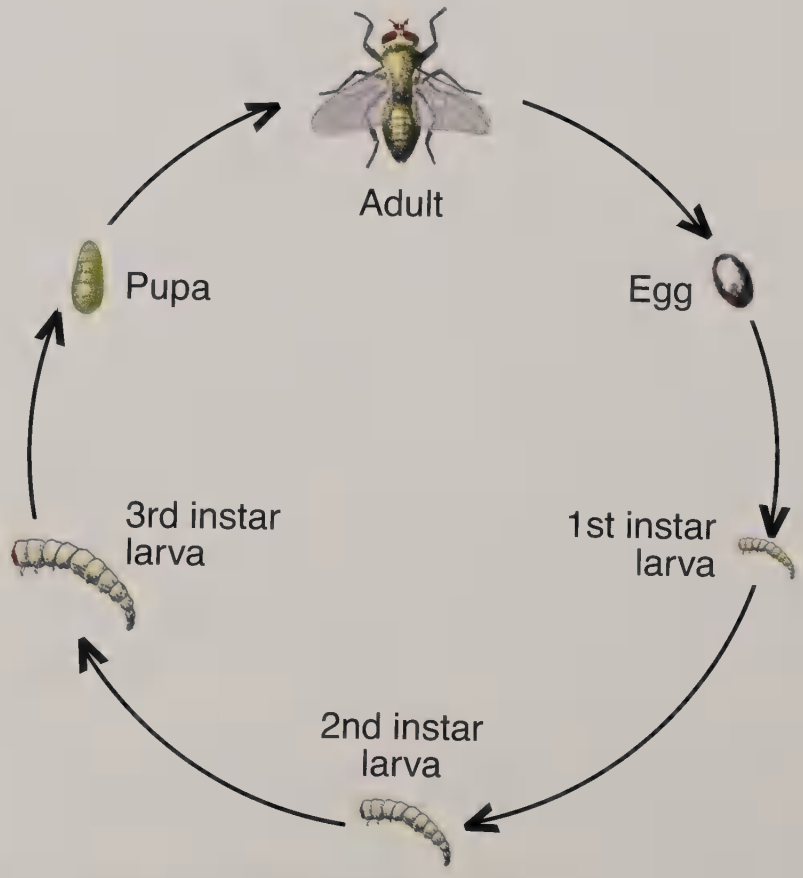


Figure 25. Example of an insect lifecycle showing complete metamorphosis.

Insects and Knapweed

Three types of insects are used in biocontrol of knapweed: flies, moths and beetles. In all, 13 species of insects, occurring in the seedheads or roots, are discussed in this manual (Table 1). All four fly species are 'fruit flies' (Family Tephritidae), in that they occur in the seedheads where the larvae eat developing flowers and seeds. One moth species and three weevil species complete the complex of 8 seed-feeding bioagents on knapweed (Fig. 26). Among the root borers are three moth species and two beetle species. One beetle, *Cyphocleonus achates*, is a weevil, and the other beetle, *Sphenoptera jugoslavica*, is a metallic wood borer.

All of the insect bioagents damage knapweed plants as larvae by feeding internally in the seedheads or roots. In general, adults have little impact on the plant except for two of the seedhead weevils, *Larinus minutus* and *L. obtusus*. Adults of these weevils can significantly defoliate knapweed stems, further weakening the plant.

Table 1 lists the natural enemies of knapweeds in the United States and the species of knapweed they attack.

It is unlikely that any one of these species alone could successfully control knapweed. Most knapweed biocontrol programs use a combination of bioagents which together create multiple stresses on the plant and have a greater chance of contributing to the suppression of knapweed.

Table 1. Knapweed bioagents established in the United States and the species of knapweed they attack.

			Knapweed Type					
	Type	Species	**Spotted**	**Diffuse**	**Squarrose**	**Meadow**	**Black**	**Brown**
Seedhead Feeders	**Flies**	*Urophora affinis*	●	●	●			
		Urophora quadrifasciata	●	●	●	●	●	●
		Terellia virens	●	●				
		Chaetorellia acrolophi	●	●	●			
	Moth	*Metzneria paucipunctella*	●	●		●		
	Beetles	*Larinus minutus*	●	●	●	●		
		Larinus obtusus	●	●	●	●		
		Bangasternus fausti	●	●	●	●		
Root Borers	**Moths**	*Agapeta zoegana*	●	●	●			
		Pelochrista medullana	●	●				
		Pterolonche inspersa		●				
	Beetles	*Cyphocleonus achates*	●	●	●			
		Sphenoptera jugoslavica	●	●	●			

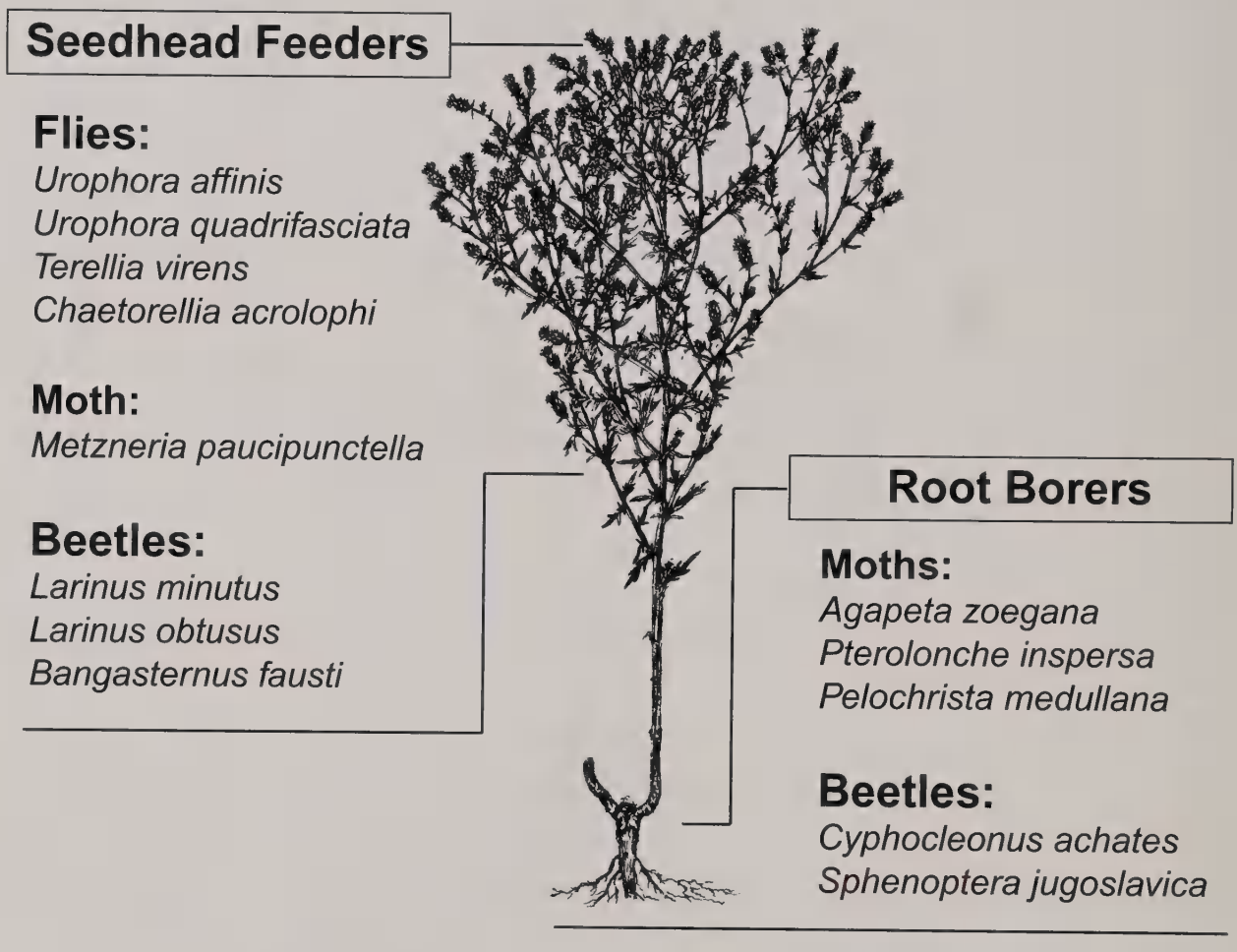


Figure 26. Distribution of knapweed biocontrol agents in a knapweed plant.

Identifying Insects

An important part of any successful biocontrol program is the ability to identify bioagents in the field. As adults, bioagents are relatively easy to identify with their variable size, color, and habits. Identifying the larvae is more challenging than the adults – and yet probably more important to know because it is in the larval stage that the bioagents: (1) do the most damage, (2) are often monitored in the field, and (3) provide conclusive evidence that the insects are established in the field.

Figure 27 is a key for identifying, in three easy steps, the larva of a fly, a moth and a beetle. This key is specific to knapweed insects, not insect larvae in general.

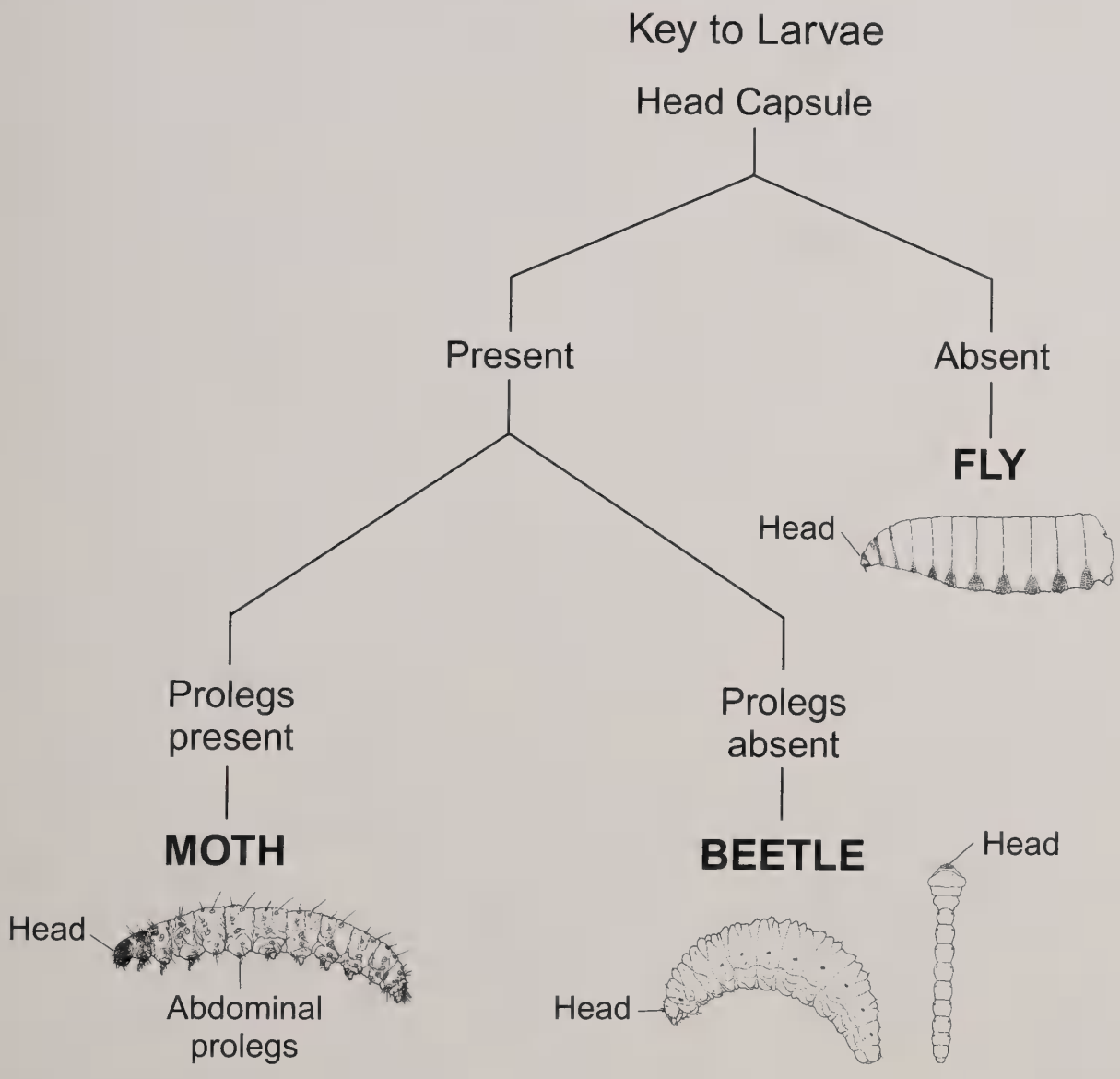


Figure 27. Key for identifying fly, moth and beetle larvae.

Fly larvae have no head capsule whereas beetle and moth larvae do. Fly larvae are sometimes confused with other larvae because they appear to have a broad, dark head. This is actually a dark, hardened anal plate anchoring the spiracles (breathing orifices).

Moth larvae have both a head capsule and prolegs.

Beetle larvae are more variable. Weevil larvae (called grubs) are white, C-shaped, and have a head capsule but no abdominal prolegs.

Figure 28 is a key for identifying the pupa of a beetle, a moth and a fly.

Beetle pupae are classified as *exarate*; they have well-developed appendages that are obviously not fused to the pupal body.

Moth pupae, with moderately developed appendages fused to the body, are classified as *obtect*.

Fly pupae are classified as *coarctate*; they are contained inside a *puparium*.

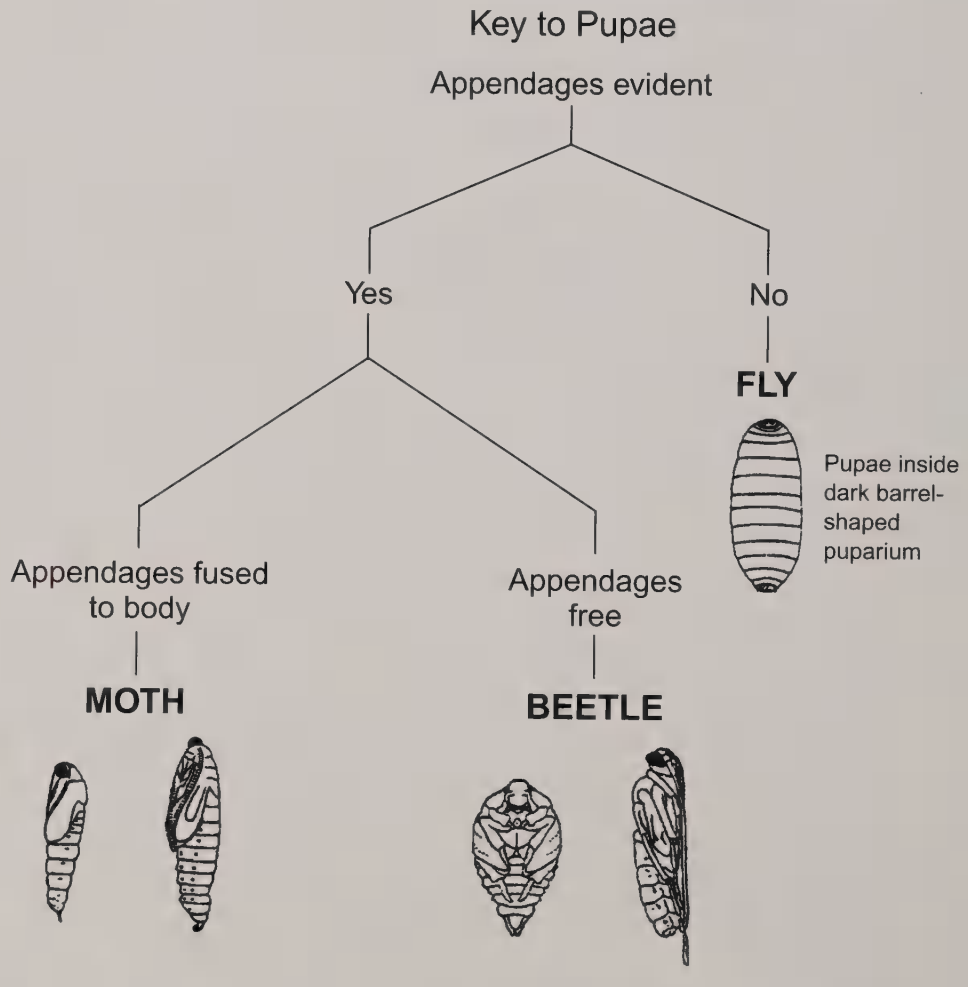


Figure 28. Key for identifying fly, moth and beetle pupae.

Seedhead Feeders

There are eight different seedhead-feeding insect species for controlling knapweeds that are established in the United States and Canada (Table 2). Among the seedhead-feeding insects are 4 fly, 1 moth, and 3 beetle species. The fly, *Urophora affinis*, was the first insect to be introduced into the United States and Canada for the biological control of diffuse and spotted knapweed. The second *Urophora* species, *U. quadrifasciata*, was not approved for release in the United States because of taxonomic concerns, but nevertheless migrated to the United States after being released in Canada. Two other flies are *Chaetorellia acrolophi* and *Terellia virens* (Fig. 29). Another seedhead feeder is the seedhead moth, *Metzneria paucipunctella*. Among the beetles are two closely related weevils, *Larinus minutus* and *L. obtusus*. The other seedhead weevil is *Bangasternus fausti*.

All of the seedhead-feeding insects damage the plant when larvae consume immature seeds and other tissues in the flower head, or capitulum. Feeding by the insects sometimes cause the plant to encase the insect larva in a hard or soft gall-like structure. In forming these galls, the weed is draining valuable nutrients away from normal plant growth (referred to as a *metabolic sink*), further depleting the plant's limited resources. Gall-forming insects are well adapted to plants like the knapweeds that produce a large number of small seedheads throughout the growing season.

Gall formers (the two *Urophora* flies) feed on actively dividing cells so they attack at the early stages of seedhead bud formation. The maximum number of gall-forming insect larvae in a seedhead is limited by the size of the seedhead, not the amount of food.

The impact that gall formers have on a plant is dictated by:

- Abundance of galls
- Power of galls as a metabolic sink
- Favorable weather conditions (i.e. drought, cold)

The other seedhead-feeding species either do not form a gall or construct a chamber in which to feed (Fig. 30). They inflict direct damage on developing seeds but do not create a metabolic sink.

Seedhead feeders are separated in time and space by such factors as:

- Type of knapweed patches insects will infest (isolated plants vs. dense stands)
- Larval feeding habits (e.g., feeding in the receptacle, the florets, the seeds)
- Number of generations per year
- Number of larvae in the head
- Overwintering site (in or out of the seedhead)

Timeline of Attack

Knapweeds produce flower heads throughout the spring and summer, creating a constant supply of seedheads of different sizes and stages of development for the seedhead feeding insects to utilize.

Figures 29 is a comparison of *U. affinis, U. quadrifasciata, C. acrolophi* and *T. virens.*

Figure 29. Comparison of Knapweed seedhead flies.

Each seedhead-feeding insect prefers certain seedhead characteristics, such as ovipositing in a certain size of seedhead (Table 3). Figure 30 shows *U. affinis* ovipositing into knapweed flower bud and the position of its eggs. Figure 31 compares the galls of *U. affinis* and *U. quadrifasciata*. Figures 32a-32b depict the position of fly, beetle and moth larvae inside the knapweed seedhead.

More than one species can occupy a seedhead at one time. This coexistence is possible because of specialized adaptations.

If the bioagent has a short adult life span, this greatly reduces the number of seedheads that the agent can attack. On the other hand, long-lived adults can attack many susceptible seedheads during its life span. Likewise, agents with more than one generation per year can capitalize on seedheads during two distinct time frames in the growing season.

Table 4 is a summary description of knapweed seedhead feeders. Table 5 compares the lifecycle of seedhead flies with the lifecycle of knapweed. Table 6 compares the lifecycle of seedhead moths with the growth stages of knapweeds.

Figure 30. *Urophora affinis* ovipositing into a closed knapweed flower bud (left) and position of eggs amid the young florets in the head (right).

Figure 31. Comparison of *U. quadrifasciata* papery gall (left) and position of larva in the hard, woody gall of *U. affinis (right).*

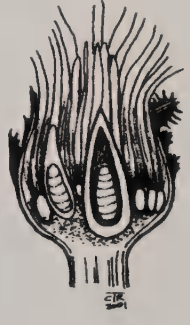


Figure 32a. Position of fly larvae inside the knapweed seedhead.

Figure 32b. Position of beetle (left) and moth (right) larvae inside the knapweed seedhead.

Table 2. List of seedhead feeding knapweed biocontrol agents.

Type	*Scientific Name*	*Common Name*
FLY	*Urophora affinis*	Banded knapweed gall fly
	Urophora quadrifasciata	UV knapweed seedhead fly
	Terellia virens	Green clearwing fly, verdant seed fly
	Chaetorellia acrolophi	Knapweed peacock fly
MOTH	*Metzneria paucipunctella*	Spotted knapweed seedhead moth
BEETLE	*Larinus minutus*	Lesser knapweed flower weevil
	Larinus obtusus	Blunt knapweed flower weevil
	Bangasternus fausti	Broad-nosed knapweed seedhead weevil

Table 3. Knapweed seedhead size and stage of development preferred by each of the eight seedhead feeding biocontrol agents.

Seedhead Development	**Seedhead Feeder**
Closed seedhead buds 0.12 inch (3mm) in diameter	*Urophora affinis* (spring only)
Seedhead buds 0.14 to 0.2 inch (4-5 mm) inch in diameter	*Bangasternus fausti* (spring only) *Chaetorellia acrolophi* (first generation spring; second generation summer)
Seedhead buds 0.2 to 0.3 inch (5 to 7 mm) in diameter	*Urophora quadrifasciata* (spring, non-obligatory second generation summer) *Chaetorellia acrolophi* (first generation spring; second generation summer)
Late seedhead bud to early bloom	*Metzneria paucipunctella* (spring only)
Full bloom	*Terellia virens* (spring flowers, if a second generation will affect attack later summer flowers)
Early seed formation	*Larinus minutus* and *L. obtusus* (adults persist for a number of weeks in the summer and lay eggs into susceptible seedheads as they become available)

Table 4. Summary of the description of knapweed seedhead feeders.

	Fly				Moth	Beetle		
Agent[1]	URAF	URQU	TEVI	CHAC	MEPA	LAMI	LAOB	BAFA
Number of Generations	One, partial second	One or two	One, partial second	Two, rarely three	One	One	One	One
Adults	Black,faint horizontal bands on wings 0.2" (5mm) long	Black, dark bands form a "UV" pattern on wings 0.2" (5mm) long	Clear-winged with yellow or greenish bodies 0.2" (5mm) long	Dark bodied with yellow bands on body and wing 0.2" (5mm) long	Gray wings folded over back when at rest 0.3" (8mm) long	Black with large snout 0.2" (5mm) long	Black, slightly mottled, bulbous snout 0.2-0.3" (5-7mm) long	Grayish-black, with blunt snouts 0.2" (5mm) long
Eggs	Cluster of 1-5 young insided unopened seedheads	Singly among developing florets	Multiple eggs laid inside the open flower head	Singly or in small clusters under bracts of flower bud	Singly on bracts at base of flower bud	Clusters are laid in the bud between pappus hairs	Singly into a newly opened head	Singly on bracts or stem leaves covered with a black egg cap
Larvae	Creamy white, barrel shaped, retracted head, circular, dark brown anal plate	Creamy white, barrel shaped, retracted head, elliptical dark brown anal plate	Barrel-shaped white, turning yellow brown	Barrel-shaped, 1st gen. white, 2nd gen. yellow	White with dark brown head capsule, five pair of prolegs	White, legless C-shaped grub with brown head capsule	White, legless C-shaped grub with brown head capsule	White, legless C-shaped grub with brown head capsule
Pupa	Inside woody gall, brown 0.06" long	Inside papery gall, brown 0.06" long	No gall, yellow-brown puparium .06" long	No gall, white puparium covered in pappus hairs	Cocoon brown append-ages fused to body	Long, white turning brown before emergence	Long, white turning brown before emergence	In a chamber in head, white (brown before emergence)
Overwinter	Larvae in seedhead	Larvae in seedhead	Larvae in seedhead	Larvae in seedhead	Larvae in seedhead	Adult in litter near root	Adult in litter near root	Adult in litter near root

Agent[1]	
URAF	*Urophora affinis*
URQU	*Urophora quadrifasciata*
CHAC	*Chaetorellia acrolophi*
TEVI	*Terellia virens*
MEPA	*Metzneria paucipunctella*
BAFA	*Bangasternus fausti*
LAMI	*Larinus minutus*
LAOB	*Larinus obtusus*

Table 5. Comparison of seedhead flies lifecycles by growth stage of knapweed plants.

	Urophora affinis	*Urophora quadrifasciata*	*Terellia virens*	*Chaetorellia acrolophi*
Knapweeds Attacked	Spotted, Diffuse	Spotted, Diffuse, Brown, Black, Meadow, Squarrose, Russian	Spotted, Diffuse	Spotted, Diffuse, Squarrose
Seedling	Overwinters as larvae in previous year's seedheads.	Overwinters as larvae or pupae in the last year's seedheads.	Overwinters as mature larvae or pupae in previous year's seedheads.	Overwinters as larvae in previous year's seedheads.
Rosette				
Bolting	Late instar larva and pupa.	Late instar larva and pupa.	Adults emerge. Mating and egg laying begin with the onset of hot, sunny weather and continues for 4-6 weeks.	
Early Flower Buds	Adults emerge and mate. Females lay eggs on young flower buds.	Adults emerge and mate.		Adults emerge and mate. Females lay eggs into flower buds.
Late Flower Buds	Larvae feed in developing seedheads. Feeding leads to development of hard, woody galls. Severely infested buds don't flower.	Egg laying between bracts of developing flower buds.	Eggs are laid in young, opening flowers. Eggs hatch in 3-5 days.	Larvae emerge from eggs and migrate to center of flower bud. Larvae pupate 10-20 days after hatch, pupating and producing a second generation. A third generation is possible, but rare.
Flowering		Eggs hatch and larvae only develop in pollinated seedheads or those attacked by *U. affinis*. Feeding leads to formation thin, papery gall.		
Seed Formation			Larvae feed for up to 14 days. 2nd generation may occur.	
Mature	10-33% of larva pupate and emerge for a 2nd generation in late forming seedheads. Majority overwinter as larvae in seedheads.	If a 2nd generation occurs, adults emerge lay eggs in susceptible seedheads. 2nd generation overwinter as larvae.	First generation larvae overwinter as pupa, 2nd generation larvae overwinter as prepupae; pupation occurs following spring.	Larvae from 2nd (possible third) generation feed upon mature seed.
Senescence				

Table 6. Comparison of knapweed seedhead moth and weevil lifecycles by growth stage of knapweed plants.

	Metzneria paucipunctella	*Bangasternus fausti*	*Larinus minutus*	*Larinus obtusus*
Knapweeds Attacked	Spotted, Diffuse.	Spotted, Diffuse.	Spotted, Diffuse, Squarrose.	Spotted, Diffuse.
Seedling	Overwinters as larvae in previous year's seedheads.	Overwinters as adults in plant litter and soil surrounding plant.	Overwinters as adults in plant litter and soil surrounding plant.	Overwinters as adults in plant litter and soil surrounding plant.
Rosette				
Bolting	Late instar larva and pupa.	Adults begin to emerge.	Adults become active feeding on leaves, including seedlings and rosettes.	Adults become active feeding on leaves, including seedlings and rosettes.
Early flower buds		Adults feed on foliage, mate, lay eggs on bracts or on end of a stem.		
Late flower buds	Adults emerge and mate, lay eggs on bracts at base of young flower heads or on stem below flower head.	Eggs hatch and larvae migrate to center of flower bud. Feed on developing florets and ovules.		
Flowering	Eggs hatch and larvae enter opened flower head, feed on florets.	Larvae complete development from egg to adult in 32 days. Adults emerge from seedhead leaving a characteristic emergence hole. Overwinter in litter and soil surrounding plant.	Mating begins.	Mating begins.
Seed formation	Larvae mine in flower base (receptacle) and feed on seeds. Overwinter as larvae in the seedhead.		Eggs laid between pappus hairs.	Eggs laid between pappus hairs.
Mature			Larvae hatch, feed on pappus hairs then move down to seeds and receptacle.	Larvae hatch, feed on pappus hairs then move down to seeds and receptacle.
Senescence			Pupate and emerge through exit holes; move to overwintering site.	Pupate and emerge through exit holes; move to overwintering site.

Urophora affinis

Order: **Diptera**

Family: **Tephritidae**

Common name: **Banded knapweed gall fly**

Weeds attacked

Spotted, diffuse, and squarrose knapweeds

Figure 33. *Urophora affinis* adult.

Description

Adult flies are about 0.2 inches (5 mm) long, black with faint horizontal bands on the wings (Fig. 33). Eggs are white when deposited, elongate and crescent-shaped. Larvae go through three larval stages or *instars*. Mature larvae are barrel-shaped, and creamy white, with heads that are slightly retracted into the thorax. A dark brown, circular anal plate develops by the end of the feeding period. The pupa is brown and 0.06 inches (3 mm) long.

Lifecycle

U. affinis usually has one only generation per year, although a small percentage of flies may undergo a second generation in late summer (August/ September). Overwintering as third instar larvae, flies pupate for about 14 days in the spring and emerge as adults at the time knapweed is in the bud stage. Emergence peaks when the largest seedhead buds are 0.12 inches (3 mm) long. Females can lay up to 120 eggs in groups of 1-5 among the immature florets inside the closed seedhead over a 3-week period. Seedheads are only susceptible to *U. affinis* oviposition for 2 to 3 days. After 3-4 days, larvae hatch from the eggs and tunnel into the base of the seedhead (receptacle). Larval feeding induces the formation of a hard woody gall, which surrounds the larva (Fig. 34). Between 10-25% of larvae pupate by 33 days and may emerge for a second generation.

Impact

Larvae directly destroy seeds. Galls drain nutrients from other parts of the plant resulting in fewer seedheads and reduced vegetative growth. Multiple galls in a single seedhead are common. The maximum number of galls that can develop in a seedhead is a function of receptacle disc area. For example, more galls are generally produced in spotted knapweed (up to 25 in one seedhead) versus diffuse knapweed which has a smaller diameter disc area. In spotted knapweed, the metabolic sinks created by *U. affinis* galls compete with root reserves so that fewer and smaller

flowering stems are produced the following year. In diffuse knapweed, each *U. affinis* gall reduced seed production by approximately 13.7 seeds and an average of 1.1 galls per seedhead reduced the above ground dry weight of the plant by 71% as well as average seed weight.

The corolla (flower petals) is suppressed or absent in heavily-galled seedheads. Woody galls can be felt when heads are rolled between the fingertips.

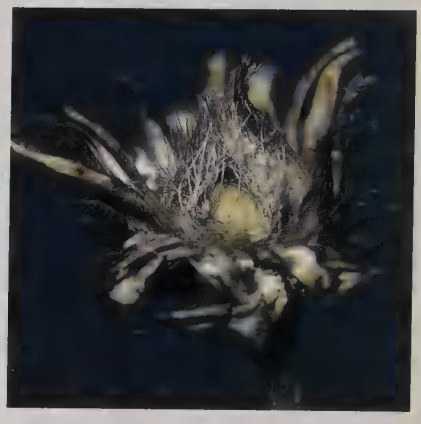


Figure 34. *Urophora affinis* larva in knapweed.

Comments

This was the first insect introduced into the United States for knapweed control. *U. affinis* does not disperse as well as *U. quadrifasciata* and other seedhead-feeding agents. On sites with both *U. affinis* and *U. quadrifasciata* infesting knapweed, *U. affinis* tends to dominate.

In Montana the combination of *U. affinis* and *U. quadrifasciata* have reduced seed production by a minimum of 50% in spotted knapweed. *U. affinis* has been found to compliment the biological control activities of *U. quadrifasciata, Metzneria paucipunctella,* and *Larinus minutus* and other seedhead-feeding agents. Studies in Canada have shown that a combination of both *Urophora* flies and the root borer *Sphenoptera jugoslavica* can reduce diffuse knapweed seed production by 98%.

Urophora quadrifasciata

Order: **Diptera**

Family: **Tephritidae**

Common name: **UV knapweed seedhead fly**

Weeds attacked

Spotted, diffuse, squarrose, meadow, black, and brown knapweeds

Description

Adult *U. quadrifasciata* flies are approximately 0.16 inches (4 mm) long, black, with black, UV pattern on the wings (Fig. 35), making this fly very easy to distinguish from *U. affinis*. Eggs are white when deposited, elongate and crescent shaped. Larvae go through three larval stages, or instars. Late instar larvae are creamy-white, barrel-shaped, with heads that are slightly retracted into the thorax. A dark brown, elliptical anal plate develops by the end of the feeding period (the anal plate of *U. affinis* is circular). Unlike *U affinis*, larval feeding causes plants to form a thin, papery gall, which surrounds the larva and is the same color as the florets. Pupae are brown, and 0.12 x 0.06 inches (3 mm) long.

Figure 35. *Urophora quadrifasciata* adult.

Lifecycle

U. quadrifasciata has at least one generation per year with a certain percentage emerging for a non-obligatory second generation. Flies preferentially attack seedheads that measure 0.22 to 0.38 inches (5 to 8 mm) long with distinct seed embryos. Eggs are laid singly among developing florets and a seedhead may be attacked several times. Eggs hatch in 3 to 4 days and larvae bore down a floret to an ovary. Larvae will only develop in pollinated seedheads. Larval feeding induces plants to form a thin papery gall around the larvae (unlike the hard gall surrounding *U. affinis*) (Fig. 36). Larvae consume most of the gall tissue during their development.

Pupation lasts about 14 days. First generation flies pupate 20-25 days after oviposition, about the time that seed development is complete. Emerging second generation adults (August) attack later forming seedheads and emerge the following spring with the onset of knapweed seedhead buds.

Impact

Florets damaged by *U. quadrifasciata* are destroyed and adjacent florets abort (approximately 1.9 seeds destroyed for each *U. quadrifasciata*). There does not appear to be a decrease in the number of seedheads on plants attacked by *U. quadrifasciata*. The fly spreads rapidly, more so than *U. affinis*. The presence of *U. affinis* in the seedhead tends to discourage *U. quadrifasciata* attack, but the combination of both fly species enhances seed reduction.

Figure 36. *Urophora quadrifasciata* larva in seedhead.

Comments

The importance of *U. quadrifasciata* will increase as knapweed densities decline because it is less dependent on dense populations of knapweed than *U. affinis*. *U. quadrifasciata* has been found to compliment the biological control activities of *U. affinis*, *Metzneria paucipunctella*, and *Larinus minutus*, and other seed head feeding agents.

On sites with both *U. affinis* and *U. quadrifasciata* infesting knapweed, *U. affinis* tends to dominate. In Montana, the combination of *U. affinis* and *U. quadrifasciata* have reduced seed production by up to 95% in spotted knapweed. Studies in Canada have shown that a combination of both *Urophora* flies and the root borer *Sphenoptera jugoslavica* can reduce diffuse knapweed seed production by 98%.

Terellia virens

Order: **Diptera**

Family: **Tephritidae**

Common name: **Green knapweed clearwing fly, verdant knapweed seed fly**

Weeds attacked

Spotted knapweed primarily, diffuse knapweed secondarily.

Description

Terellia virens is a soft seed feeder like the *Larinus* species. This fly does not form galls. Adults are about 0.2 inches (5 mm) long, greenish-gray flies with wings (Fig. 37). Eggs are elongate, about 0.04 inches long (1 mm), and shiny white. Young larvae are white, but turn yellow-brown as they mature. Pupae are yellow-brown.

Figure 37. *Terellia virens* adult.

Lifecycle

Weather conditions determine the number of generations (one or two). If there is only a single generation, flies spend the winter as pupae in the seed head oriented vertically above the receptacle in a loose cocoon of plant hairs. With two generations, flies spend the winter as mature larvae in cocoons partially embedded in the flower base (receptacle).

Adult *T. virens* begin to emerge in late May, about 4 weeks before spotted knapweed flowers. Mating and oviposition begin with the onset of warm weather and continues for the length of the adult's life. The adult flies live for about 48 days.

Females lay eggs in young, opening flowers heads from early June to early October. After laying one to several eggs into the flower heads between the flowers, the female marks the bracts of the head and upper stem leaves with a substance to discourage oviposition by other females. Each female will lay an average of 80 eggs that hatch within 3 to 5 days.

Larval development to pupation takes about 14 days. The barrel-shaped larvae spend their first two instars inside a single seed, feeding on ripening seed. Two to several *T. virens* larvae may infest a seedhead.

Impact

T. virens larvae cause considerable destruction of seeds; partial feeding damage on other seeds can reduce viability of the remaining seeds by up to 90%.

Comments

This fly can co-exist in seedheads infested by *Chaetorellia acrolophi* and *Urophora* species but is a poor competitor in heads infested by *Larinus* species. *T. virens* prefers plants on south-facing slopes and dry locations. It is established in many states and is most successful in areas without *Larinus* species. In Montana, this fly appears to be severely hindered by high densities of *Urophora affinis*.

Chaetorellia acrolophi

Order: **Diptera**

Family: **Tephritidae**

Common name: **Knapweed peacock fly**

Weeds attacked

Spotted and diffuse knapweeds.

Description

C. acrolophi is an ovary feeder. Adults are small, 0.2 inches (5 mm) in size, yellow-brown flies with bright green eyes and light-brown wing bars (Fig. 38). Eggs are shiny white, elongate, and have a long filament thickened at one end. Larvae are white and develop through three instars. Pupae are contained within a white puparium.

Figure 38. *Chaetorellia acrolophi* adult.

Lifecycle

C. acrolophi generally has two generations a year; a third generation is possible but rare. Adults emerge in early June when knapweed plants are in the bud stage. Mating begins immediately and oviposition lasts for the remainder of the 17-day lifespan of the adult female. Eggs are laid singly or in batches of two to four underneath the bracts of unopened buds. A female will lay an average of 69 eggs in its lifetime. Larvae hatch 4 to 5 days later and migrate into the center of the flower buds where they feed on immature florets as they descend to the seeds. Second and third instar larvae feed on developing seeds, florets, and partially on the receptacle. Larvae pupate 10 to 15 days after hatching. First generation adults generally emerge in July, mate and lay eggs, which develop into the second generation.

First generation larvae and pupae are white and pupae are enclosed in a white pupal case covered in pappus hairs from the seeds. Second generation larvae and pupae are tan-colored, with pupae enclosed in a yellow puparium covered with pappus hairs from the seeds. Second generation larvae typically overwinter in the flower heads, then pupate the following spring.

Impact

This fly does not cause plants to form galls. Larval feeding can significantly reduce seed production; a single larva can consume the entire contents of a single seedhead.

Comments

Chaetorellia acrolophi prefers plants in moist habitats and is generally associated with scattered plants rather than in dense stands of spotted knapweed. It is established in many states and, in Oregon, is most successful in areas where *Larinus* species are not present. This fly should supplement the impact of *U. affinis* by attacking isolated knapweed plants; however, in Montana it appears to be hindered by high densities of *U. affinis*. This fly is not widely distributed but is established in Oregon and Montana.

Metzneria paucipunctella

Order: **Lepidoptera**

Family: **Gelechiidae**

Common name: **Knapweed seedhead moth**

Weeds attacked

Spotted knapweed preferred, will attack diffuse knapweed.

Description

Metzneria paucipunctella is a small moth, 0.32 inches (8 mm) long. Adults fly at dusk and are rarely seen. The adult's front wings are light gray with peppery spotting and dark at the tip, and when at rest, folded over the back (Fig. 39). The eggs are elongate, oval, and reddish-brown when first deposited but turn yellowish as they mature. Larvae are 0.16 to 0.20 inches (4 to 5 mm) long, white with dark brown head capsules and several pairs of prolegs. Pupae, enclosed in a cocoon, are brown with appendages fused to the body.

Figure 39. *Metzneria paucipunctella* adult.

Figure 40. *Metzneria paucipunctella* larva in a knapweed seedhead.

Lifecycle

M. paucipunctella has one generation per year. The gray adults begin to emerge in late May and immediately begin mating. Each female moth may lay from 60 to 100 eggs in a three-week period, beginning in June. Eggs are placed singly on the bracts at the base of the young flower heads, or on the stems just below the flower head.

Larvae hatch in 10-12 days as the flower heads are opening. Larvae enter the opened flower heads; first instar larvae feed on the florets while the second-instar larvae feed on the seeds. Third instar larvae mine into the flower base, which reduces the viability of uneaten seeds. Several young larvae can occupy a seedhead early in the season but only one larva survives beyond the third instar (Fig. 40). In the fall the moth larva moves from the receptacle to

overwinter in the base of the seedhead. Pupation occurs in the spring and lasts for 3 to 4 weeks.

Impact

Larvae feed on developing seeds. Each larva can destroy on average of 8 seeds and reduce the viability of others. Older larvae web seeds together preventing seeds from dispersing over long distances. Older larvae will eat *Urophora* larvae.

Comments

M. paucipunctella can suffer severe mortality during cold winters. Moth feeding compliments the biological control caused by *Urophora* species. *M. paucipunctella* larvae are aggressive and will kill one another or other knapweed seedhead-infesting larvae.

Larinus minutus

Order: **Coleoptera**

Family: **Curculionidae**

Common name: **Lesser knapweed flower weevil**

Weeds attacked

Diffuse and spotted knapweed; has become established on squarrose knapweed in California.

Description

Larinus minutus is a small black weevil and a soft seed-feeder like *Terellia virens* and *Larinus obtusus*. Adults are 0.16-0.2 inches (4 to 5 mm) long, black and have a short robust snout (Fig. 41). Eggs are elongate, yellow and are often clustered in the seed head between pappus hairs. Larvae are white, legless, C-shaped grubs with brown head capsules which go through three larval instars and reach a length of approximately 0.3 inches (8 mm) in length. Pupae are 0.24 inches (6 mm) long, and white, turning brown shortly before emergence and generally resemble the adult weevil.

Figure 41. *Larinus minutus* adult.

Lifecycle

L. minutus has one generation per year. Adults spend the winter in plant litter within the infestation. Adults are active in the field from May or June until August. Mating occurs continuously during this 11-week period. Adults feed on the leaves (including rosette leaves in the spring) and flowers prior to laying eggs. Eggs are deposited in the seedhead between the pappus hairs. Up to five eggs are clustered; the number of eggs laid per female ranges between 28 and 130. Eggs hatch three days later and the newly hatched larvae feed on the pappus hairs, then move downward to consume seeds and partially the receptacle. Feeding lasts about four weeks as larvae go through three instars. The number of *L. minutus* larvae a seedhead can support depends on the size of the seedhead and the knapweed species. The larva constructs a pupae chamber (partly from seed coats) attached to the flower base in which to house the pupa. New adults emerge and feed on foliage and flowers before moving to overwintering sites at the base of the plants.

Impact

Adult feeding can severely defoliate plants (Fig. 42). Larval feeding reduces seed production; a single larva can destroy the contents of an entire diffuse knapweed seedhead.

Emerging adults make characteristic emergence holes in the center of affected seedheads similar to the emergence holes created by *B. fausti* and *L. obtusus* (Fig, 43).

Figure 42. Defoliation of knapweed by *Larinus minutus* adult.

Comments

L. minutus larvae are aggressive and will kill one another or other insects in the same seedhead.

L. minutus was established in 1998 on squarrose knapweed in California.

Population increases of *L. minutus* on spotted knapweed have been slow; however, it still appears to be a very promising agent. The insect can have a significant impact on the plant growth and density across a wide range of habitats.

A study in Minnesota found that reduction in spotted knapweed infestation increased by 26.5% with the addition of *L. minutus* to existing *U. affinis* and *U. quadrifasciata* populations. The number of seeds destroyed in individual seedheads increased. *L. minutus* and the two *Urophora* species were found to successfully cohabit in spotted knapweed seedheads.

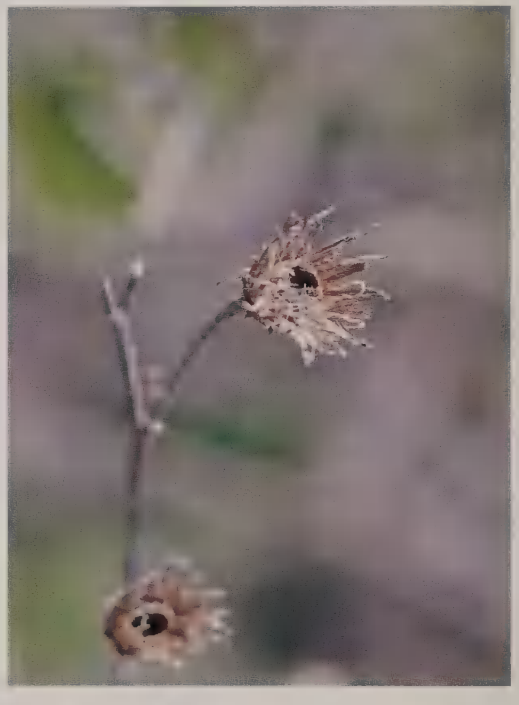


Figure 43. *Larinus minutus* emergence hole.

In addition to seed destruction by larvae, adults can do extensive damage by feeding on growing plants in the spring, which often results in the near total destruction of all growing diffuse knapweed plants in the vicinity of the original insect release. Diffuse knapweed plants under attack by *L. minutus* typically turn a characteristic blue-green color, have few leaves and often have distorted growth. Adult *L. minutus can* also destroy diffuse knapweed seedling, resulting in suppressed recruitment of new plants. The insects develop large populations within 3 to 5 years and disperse rapidly to new areas.

Larinus obtusus

Order: **Coleoptera**

Family: **Curculionidae**

Common name: **Blunt knapweed flower weevil**

Weeds attacked

Spotted is preferred and to a lesser extent diffuse knapweed

Description

Larinus obtusus is a close relative of *L. minutus*. It is a small black weevil measuring 0.20 to 0.28 inches (5 to 7 mm) long; black with a somewhat mottled appearance caused by patches of white hair on their back, and a prominent, bulbous snout (Fig. 44). It too is a soft seed feeder. Eggs are yellowish, oval to round. Larvae are 0.3 inches (8 mm) long, white, legless, C-shaped grubs with brown head capsules. Pupae are 0.24 inches (5 mm) long, white turning brown shortly before emergence.

Figure 44. *Larinus obtusus* adult.

Lifecycle

L. obtusus has one generation per year. Adults spend the winter in soil litter at or near the base of plants. Overwintering adults appear at the end of May and reach peak population levels during early July. Adults feed heavily on the foliage and flowers prior to mating and laying eggs. Females oviposit throughout their 5 to 6 month lifespan among the inner florets of newly opened flower heads. Occasionally adults may hibernate a second time and live a second season.

Eggs hatch in 3 to 6 days and larvae begin feeding on pappus hair and developing seeds. More than one larva can occupy a seedhead. Larvae develop through three instars over a 4 to 6- week period, pupating in chambers constructed from cemented seeds and pappus hairs. The pupal period generally lasts 9 days. Adults emerge late July and early August through holes chewed in the tops of the pupal chambers and vigorously feed on foliage before moving to overwintering sites in the soil.

Impact

One or two larvae can destroy most of the developing seeds in the head. Any seeds not eaten become part of the pupal chamber. Adult feeding on foliage can reduce photosynthetic capacity and plant vigor.

Emerging adults make characteristic holes in the center of affected seed heads, similar to the emergence holes created by *B. fausti* and *L. minutus*.

Comments

L. obtusus prefers moist sites in contrast to the other seedhead weevils for knapweed, which prefer and thrive in drier sites. It has not yet been established on knapweed species other than spotted in the United States. *L. obtusus* has been slow to build up significant populations in spotted knapweed in western Montana.

Bangasternus fausti

Order: **Coleoptera**

Family: **Curculionidae**

Common name: **Broad-nosed knapweed seedhead weevil**

Weeds attacked

Spotted, diffuse, and squarrose knapweed

Description

Bangasternus fausti is a small, gray-brown weevil measuring 0.16 inches (4 mm) with a short, blunt snout (Fig. 45). Eggs are oval, yellowish, and covered with a black egg-cap. Larvae are white, legless, C-shaped grubs with brown head capsules that reach a length of approximately 0.3 inches (8 mm). The white, 0.24 inches (5 mm) long pupae are found inside a cell in in the seedhead.

Figure 45. *Bangasternus fausti* adult.

Lifecycle

B. fausti has one generation per year. Adults spend the winter in plant litter and soil surrounding the plant (in warmer climates, adults overwinter in the seedheads). Adults become active in May and begin mating. They feed on knapweed foliage in the spring and on flowers in the summer. Eggs are laid individually on the seedhead bracts or on the end of the stem and leaflets from May to mid-August. Eggs are covered with a black egg-cap and hatch in 8 to 12 days.

Depending on the placement of the egg, the new larva mines directly into the bud or into the stem and then tunnels to the bud where it feeds within the seedhead. Pupation occurs in the damaged head within a cell constructed by the larva of frass and fused seeds. It takes approximately 32 days for *B. fausti* to go from egg to adult. Adult *B. fausti* feed on knapweed foliage in the spring and on flowers in the summer.

Impact

B. fausti feeds in the flower base and destroys the flowers and ovules before they produce seeds. Weevils can consume 95-100% of the seed. In the fall, attacked seedheads have a characteristic emergence hole similar to emergence holes of *Larinus* species.

Comments

B. fausti has become well established on spotted knapweed in the United States. It is not known how *B. fausti* will interact with other seedhead- infesting biological control agents. Early concerns about the potential of *B. fausti* to displace *Urophora affinis* have yet to be realized. Under favorable conditions weevil density can increase dramatically allowing the collection of large numbers of weevils for collection and redistribution (Fig. 46).

Figure 46. *Bangasternus fausti* being released on spotted knapweed.

Root Borers

There are five root boring insect species established in the United States and Canada for the control of diffuse, spotted and squarrose knapweeds. Three species are moths (sulfur knapweed root moth, *Agapeta zoegana*; brown-winged knapweed root moth, *Pterolonche inspersa*, and gray-winged knapweed root moth, *Pelochrista medullana*), and two are beetles (knapweed root weevil, *Cyphocleonus achates*, and bronze knapweed root borer, *Sphenoptera jugoslavica*. All these insects can be present in the root at the same time. Studies are underway to determine how these insects coexist and compete in knapweed roots.

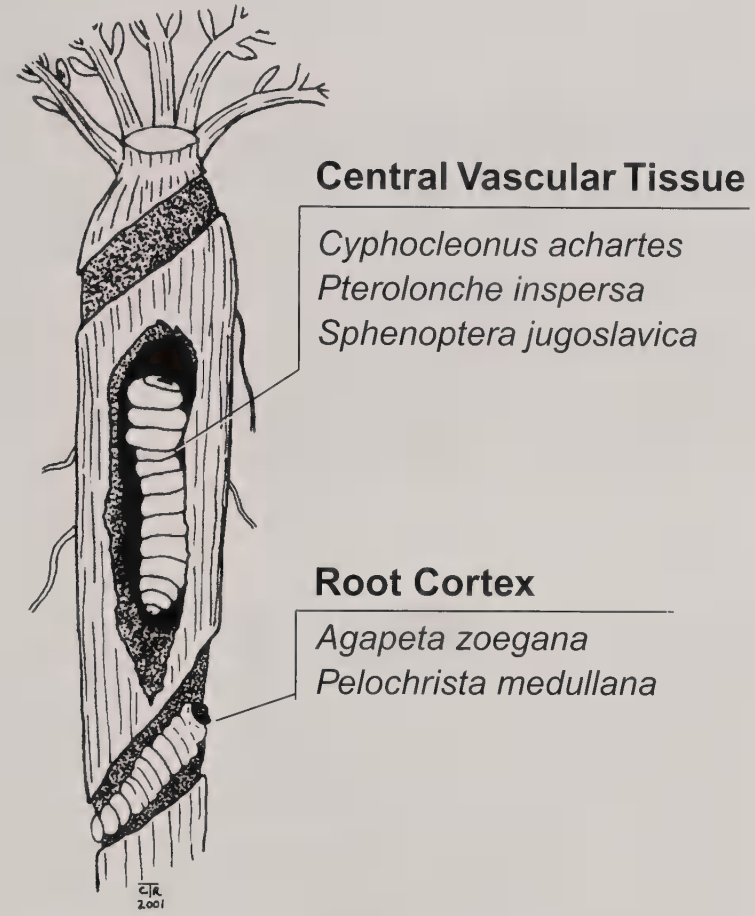


Figure 47. Distribution of knapweed root borers.

All five root-feeding insects damage the plant in the larval stage by feeding on the central vascular tissue or the cortex of the root just below the epidermis, depending on species (Fig. 47). Eggs are laid on the stem, on the basal rosette leaves, on the soil surface, or on the root crown just below the soil surface. Upon emerging from the eggs, larvae immediately burrow into the root, where they feed and complete their development.

As larvae, insects mine the roots depleting the carbohydrate reserves of the plant that are important for growth and essential for overwintering. In addition to mining the roots, beetle larvae of *S. jugoslavica* and *C. achetes* cause root galls. Galls create a metabolic sink, meaning that the energy generated by the plants through photosynthesis is used to produce the gall rather than to meet critical plant needs.

All the insects are *univoltine*, which means they produce only one generation per year. Most larvae complete their development in a single root; however, larvae of the sulfur root moth, *Agapeta zoegana*, can migrate a short distance between roots during the growing season.

TYPE	SCIENTIFIC NAME	COMMON NAME
Moth	*Agapeta zoegana*	Sulfur knapweed root moth
Moth	*Pterolonche inspersa*	Grey-winged knapweed root moth
Moth	*Pelochrista medullana*	Brown-winged knapweed root moth
Beetle	*Cyphocleonus achates*	Knapweed root weevil
Beetle	*Sphenoptera jugoslavica*	Bronze knapweed root borer

Agapeta zoegana

Order: **Lepidoptera**

Family: **Cochylidae**

Common name: **Sulfur knapweed root moth**

Weeds attacked

Spotted knapweed primarily, diffuse and squarrose knapweed secondarily.

Description

Agapeta zoegana is a small, bright-yellow moth, 0.44 inches (11 mm) long, with brown wing bands (Fig. 48). Adults may be found resting vertically on the knapweed stems or under the leaves. Eggs are white, turning orange, round but somewhat flattened (Fig. 49). Larvae are white and have a brown head capsule and legs, and about 0.3 inches (7 mm) long (Fig. 50). Pupae are white.

Figure 48. *Agapeta zoegana* adult.

Figure 49. *Agapeta zoegana* egg at the base of knapweed rosette leaf.

Lifecycle

Adult moths emerge from overwintering as larvae in knapweed roots in early July through early September. Mating takes place within 24 hours after emergence and the mated female begins ovipositing eggs the next day, laying eggs in the stem crevices and on the leaves of knapweed plants. Eggs are laid singly or in groups of 2-3. Adults live 11 to 14 days with each female laying from 21 to 78 eggs in her lifetime. The larvae hatch from the eggs in 7 to 10 days and move immediately to the root cortex. Larvae develop through six instars with mature larvae overwintering in the root and pupating early the next summer.

Impact

There can be multiple larvae in the roots. Larvae are mobile and can migrate a short distance to other plants. Larval feeding can kill young plants; larger plants often do not flower.

Comments

A. zoegana was first released in the United States in 1984 and is now established in most western states. A pheromone (chemical attractant) system has been developed to monitor this moth (see "Monitoring Biocontrol Agents" in Chapter 3).

Figure 50. *Agapeta zoegana* larva in the root.

Pterolonche inspersa

Order: **Lepidoptera**

Family: **Pterolonchidae**

Common name: **Grey-winged knapweed root moth**

Weeds attacked

Diffuse knapweed

Description

Pterolonche inspersa adult is a light-brown moth with a 0.8 inch (2 cm) wingspan and 0.3 inch (7 mm) body length. There are no distinct markings on the wings (Fig. 51). The eggs are oval and black.

Lifecycle

P. inspersa produces one generation per year. Adults emerge from June to early September, mate and lay eggs during their short, 15 to 20-day life span. Eggs are laid singly or in small groups on the undersurface of rosette leaves. Upon hatching, the larvae tunnel into the root crown and begin to feed on root tissue. As they reach the root cortex, they spin a silken tube and feed from within the tube. Mature larvae overwinter in the roots of the knapweed plants. In the spring they spin a silken tube 0.8 inches (2 mm) above the soil surface to pupate and provide an easy exit for the emerging adult.

Figure 51. *Pterolonche inspersa* adult.

Impact

Infested diffuse knapweed plants can be recognized in the spring by the silken tubes around the crown of the rosette. *P. inspersa* larvae cause considerable root damage and as a result, plants attacked by the larvae are stunted and produce fewer flowers. The infested root becomes spongy and easy to pull from the ground. Feeding damage reduces root storage.

Comments

P. inspersa, a native moth of Europe, was approved for release in 1986. *P. inspersa* larvae are known to eat the larvae of the bronze knapweed root beetle, *Sphenoptera jugoslavica.* It is difficult to establish this moth, and is reportedly established only in Montana.

Pelochrista medullana

Order: **Lepidoptera**

Family: **Tortricidae**

Common name: **Brown-winged knapweed root moth**

Weeds attacked

Spotted and diffuse knapweed

Description

Pelochrista medullana is a tan to gray moth with mottled wings measuring 0.4 inches (10 mm) long (Fig. 52). Eggs are oval, flattened and ribbed.

Figure 52. *Pelochrista medullana* adult.

Lifecycle

P. medullana produces one generation per year. Adults emerge mid-June to late July to mate (within 24 hours after emergence) and lay eggs. Adults live about 2 weeks. Eggs are laid primarily on the lower surface of rosette leaves. Females can lay up to 120 eggs in warm dry weather but this can be greatly reduced by cold, rainy weather.

Larvae hatch 7 to 9 days after oviposition and move to the center of the rosette and mine into the root crown. Larvae mine spiraling tunnels around the cortex of the root, just under the epidermis, similar to *Agapeta zoegana*. The tunnels are lined with a silken web. Larvae overwinter in the roots and complete development in the spring or early summer. Usually only one larva is found on an infested plant.

Impact

Damage to the roots is similar to that caused by *Agapeta zoegana*. Only 3rd to 6th instar larvae cause measurable damage, reducing root storage capacity and exposing the plant to pathogens. Small plants, <0.4 inch (10 mm) root diameter, can be completely destroyed. Plants that survive insect attack are usually smaller and produce fewer flower heads than uninfested plants.

Comments

P. medullana is a root-boring moth native to central Europe approved for release in the United States in 1984. Limited numbers of *P. medullana* have been released in Idaho, Montana, Oregon and British Columbia. However, to date, there is no evidence of establishment of this agent in the United States or Canada.

Cyphocleonus achates

Order: **Coleoptera**

Family: **Curculionidae**

Common name: **Knapweed root weevil**

Weeds attacked

Spotted knapweed preferentially, diffuse and squarrose knapweed secondarily.

Description:

Cyphocleonus achates is a large, 0.5 to 0.6 in (13 to 15 mm) long, brown-gray mottled weevil with a short, thick snout (Fig. 53). Eggs are oval, cream-colored and noticeable on the plant. Larvae are white, C-shaped grubs with a brown head capsule, and about 0.5 inches (13 mm) long.

Figure 53. *Cyphocleonus achates* adult.

Lifecycle

This weevil has one generation per year. Adults emerge from mid-July to early September with peak emergence at about mid-August. Adults spend most or their short life (about 10 weeks) on the root crown, just below the surface. They climb up to the tops of plants on sunny, warm days in search of a mate. Larvae hatch in 10 to 12 days and begin to tunnel into the root central vascular tissue where they will complete their development. Weevils pupate inside the root (Fig. 54) *C. achates* overwinters as young larvae in the root. Mature larvae can cause a gall to form in the root giving the root a swollen appearance. They pupate in the root gall with the onset of warmer spring temperatures. New adults appear after about two weeks of pupation by chewing their way out of the root. Evidence of larval damage is a wide tunnel, abundant frass (insect excrement), and a swollen root gall (caused by the third and fourth instar). Unlike other knapweed beetles, *C. achates* has four larval instars. By the fourth instar, larvae are large, white and obviously C-shaped.

Figure 54. *Cyphocleonus achates* pupa in knapweed root.

Impact

Small plants can be killed as a direct result of larval feeding. Most damage is done when multiple larvae occupy a root or when the attacked roots are small. Older larvae cause a gall to form in the root, which acts as a metabolic sink. Plants are stunted and some survive only one season after being infested with *C. achates*. Tunneling in the root also exposes the plant to bacterial and fungal infection that can cause additional secondary injury.

Comments

This root-boring weevil was first released in the United States in 1988 and is now established in several states. *C. achates* typically does not fly and consequently has been slow to establish and spread. In hot weather adults can be seen on the tops of the plants. Its habit is to sit perfectly still and when disturbed, to drop to the ground and play dead. Up to 25 larvae have been recorded in the same root. This is probably the best knapweed root-boring bioagent available today. Techniques have been developed to mass-rear this insect for greater production and more rapid distribution.

Sphenoptera jugoslavica

Order: **Coleoptera**

Family: **Buprestidae**

Common name: **Bronze knapweed root borer**

Weeds attacked

Diffuse primarily, spotted secondarily.

Description

Sphenoptera jugoslavica adults are about 0.4 inches (10 mm) long, bronze-colored and somewhat flattened (Fig. 55). Eggs are flat and change color from white when first laid, to dark bluish -purple after about five days (Fig. 56). Larvae have an enlarged head and a long thin cylindrical body tapering at the end (Fig. 57). Pupae are initially white, but later darken.

Figure 55. *Sphenoptera jugoslavica* adult.

Lifecycle

S. jugoslavica has one generation per year. Adults emerge in mid to late July. They feed on knapweed leaves for two to three days before mating. Females lay multiple eggs during July and August between the base of rosette leaves. Larvae hatch from the eggs and begin to tunnel into the root's central vascular tissue where they will complete their development through three instars. *S. jugoslavica* overwinters as larvae in the root. Larvae pupate in the root gall with the onset of drier conditions and warm temperatures. There can be multiple larvae in the roots. Evidence of larval damage is a wide tunnel, abundant frass, and a root gall.

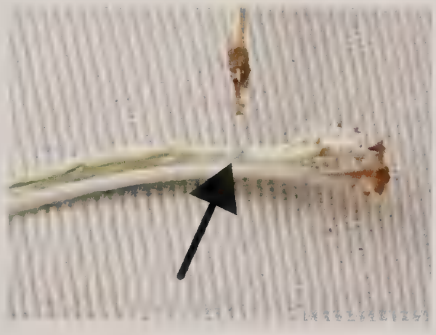


Figure 56. *Sphenoptera jugoslavica* egg.

Impact

Larvae mining the roots can cause significant impact; adult feeding on the leaves is much less damaging. The larvae cause a gall-like swelling in the knapweed root near the crown. The depletion of root carbohydrates can kill the plant or retard rosette growth. Attacked plants are often stunted and produce fewer seeds the following season.

Figure 57. *Sphenoptera jugoslavica* larva in a knapweed root.

This beetle prefers hot, dry sites typical of those infested with diffuse knapweed, but will also establish on drier spotted knapweed sites.

Comments

S. jugoslavica, was first released in the United States in 1979 and is now widely established in the West. It performs best in hot, dry diffuse knapweed sites with shallow, stony soil.

Table 7. Summary description of knapweed root borers.

	Moth			Beetle	
Agent[1]	**AGZO**	**PTIN**	**PEME**	**CYAC**	**SHJU**
Generations	One	One	One	One	One
Adults	Bright yellow moth with brown wing bands. 0.44" (11mm) long	Light brown moth with tan-colored wings. 0.8" (2cm) long	Tan to gray with mottled wings. 0.3" (8mm) long	Large, mottled gray color. 0.4- .6" (30-40 mm) long	Bronze metallic-colored, elongate and flattened. 0.4" (10mm) long
Life span	11 to 14 days	About 15 days	2 weeks	10 weeks	12 weeks
Eggs	Eggs are white, turning orange, round and ribbed, laid singly or in groups of 2-3 in stem and leaf crevices.	Eggs are oval and black, laid singly or in groups pf 2-3 on underside of leaf.	Eggs are oval, white and flattened, and ribbed. Laid singly or in small batches on the underside of leaf surface.	Eggs are oval and cream-colored, laid in batches on the root crown, just below the soil surface.	Oval, flattened, white turning bluish-black, laid on the base of one rosette leaf petiole.
Larvae	Larvae are white and have brown head capsule and legs.	Larvae are elongate, pale and have head capsule and legs. Found in a silken cocoon.	Larvae are elongate, pale and have head capsule and legs. Found in a mined tunnel in silken web.	Larvae are large, C-shaped grubs located inside a gall in the center of the root 0.1" (2.5mm) long.	Cylindrical, tapering at the tail end. 0.4" (10mm) long.
Pupa	Large, white, appendages fused to body.	Large, white, appendages fused to body.	Large, white, appendages fused to body.	Large, white, with free appendages.	Large, cream-colored with free appendages.
Overwinter	Mature larvae in the root.	Mature larvae in the root.	Mature larvae in the root.	Mature larvae in the root.	Mature larvae in the root.

Agent[1]
AGZO *Agapeta zoegana*
PTIN *Pterolonche inspersa*
PEME *Pelochrista medullana*
CYAC *Cyphocleonus achates*
SHJU *Sphenoptera jugoslavica*

Table 8. Comparison of knapweed root borer lifecycles by knapweed growth stage.

	Agapeta zoegana	*Pterolonche inspersa*	*Pelochrista medullana*	*Cyphocleonus achates*	*Sphenoptera jugoslavica*
Seedling	Overwinters as larvae in previous year's roots.	Overwinters as larvae in previous year's roots.	Overwinters as larvae in previous year's roots.	Overwinters as larvae in previous year's roots.	Overwinters as larvae in previous year's roots.
Rosette					
Bolting	Larvae pupate and new adults emerge.	Larvae pupate and new adults emerge.	Larvae pupate and new adults emerge.	Larvae pupate and new adults emerge.	Larvae pupate and new adults emerge.
Early flower buds					
Late Flower Buds	Mating; eggs laid at base of basal leaves.				
Flowering	Larvae hatch and chew into root cortex.	Adults mate and females lay eggs.	Adults mate and females lay eggs.	Adults mate and females lay eggs.	Adults mate and females lay eggs.
Seed formation	May migrate to other nearby roots and continue development.	New larvae migrate to root vascular tissue.	New larvae migrate to root cortex.	Larvae burrow into root central vascular tissue, forming a gall in the root.	Larvae burrow into root central vascular tissue, forming a gall in the root.
Mature					
Dissemination	Overwinter	Overwinter	Overwinter	Overwinter	Overwinter

Chapter 3
Elements of a Knapweed Biological Control Program

This chapter discusses the elements necessary to successfully establish a knapweed biological control program. Biological control programs require years of continuous observations and a commitment to specific steps or processes.

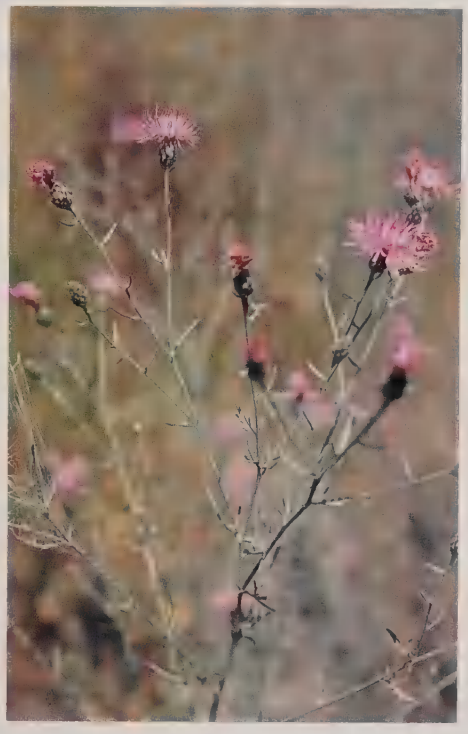


Figure 58. Spotted knapweed plant.

To successfully establish a knapweed biological control program, follow these guidelines:

1. **Background information** - Read the information contained in this manual and become familiar with: (a) general knowledge of biological control of weeds, (b) knapweed species, and (c) their biocontrol agents (also referred to as bioagents - species of flies, moths, and beetles, which are the natural enemies of knapweeds). An ability to identify knapweeds (Fig. 58) by appearance and growth stage, each of the biocontrol agents, and what they do to the weed, is essential.
2. **Select the release site** - Make note of the bioagents already present at the selected site (see "Selecting a Study or Nursery Site").
3. **Schedule field activities** - Timing of the collection and release is crucial in the success or failure of a biocontrol program; thus, pay close attention to scheduling of field activities. For optimum results, follow the timetables suggested in this chapter as closely as possible.
4. **Obtain bioagents** - Obtain and release the natural enemies at the selected site.
5. **Monitor bioagents and vegetation** - Monitor bioagents (see "Monitoring Biocontrol Agents"), and vegetation (see "Monitoring Vegetation").

For solutions to common problems encountered when establishing a biocontrol program, see Appendix A: Troubleshooting Guide: When Things Go Wrong.

A systematic process to establish a knapweed biological control program consists of the following elements:

1. Selecting and Preparing Study Sites
2. Collecting Biocontrol Agents
3. Transporting Biocontrol Agents
4. Releasing Biocontrol Agents
5. Monitoring Biocontrol Agents
    a. Monitoring Vegetation (quantitative and qualitative)
    b. Establishing Photo Points

Methods for carrying out each of these processes are discussed in separate sections in this chapter.

1. Selecting and Preparing Release Sites

Three types characterize a biocontrol program site: (1) study and (2) nursery, and (3) field release site.

Study site. A study site is a release site where the damage and impact is established. Study sites can be used as demonstration areas for educational and training purposes, and can be monitored intensively to determine the effects of bioagents on knapweed over time. However, monitoring activities should be planned carefully because frequent site visits can damage the site through disturbance and trampling of vegetation.

Nursery site. A nursery site, or field insectary, is used for collecting adequate quantities of bioagents for redistribution to other knapweed infested areas where bioagents have not been previously released or are of low quantity. Nursery sites should be left undisturbed for 3-5 years to allow the bioagent populations to increase. Monitor nursery sites occasionally to determine if the released bioagents have become established. After three years, bioagent populations may be large enough to enable collection and redistribution. It is essential to select nursery sites which offers minimal disturbance.

Field Release. A field release site is simply an open site for general control purposes. It is not intended for specific monitoring or redistribution.

Selecting the Site

The type of site you select will depend on the objectives of your biocontrol program. Use the following guidelines and criteria to select a site (study, nursery, or field release):

Visit prospective sites – Visit several prospective release sites. Useful criteria for determining the suitability of a site include:

Presence of bioagents: If bioagents are already present at the prospective site, move on and choose a different location.

Size of site: An area with at least 2 acres of knapweed infestation is preferable however, a larger area of infestation is more desirable (Fig. 59).

Location: Consider accessibility, slope and cover (avoid shaded, forested sites).

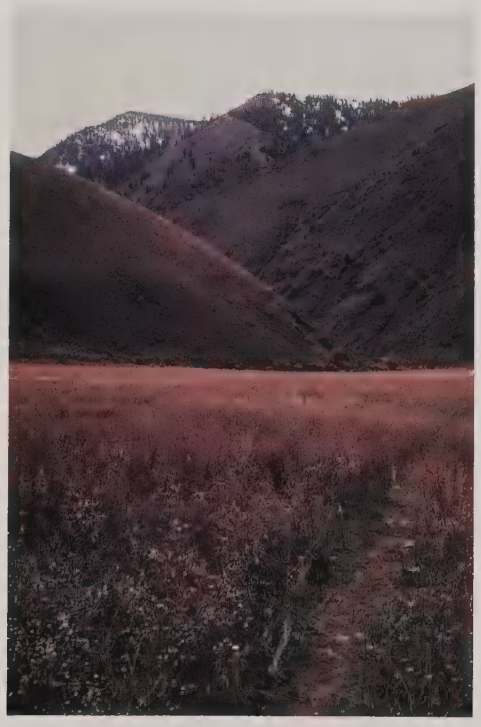


Figure 59. An example of a spotted knapweed infestation suitable for a biocontrol program.

Density of infestation: Choose a moderately dense area of infestation, an area containing three or more knapweeds per square yard.

Disturbance: Select sites that are not cultivated, away from land development, and where no livestock are grazed.

Pesticides: Select sites which are pesticide-free (no herbicides and insecticides have been applied to the area).

Obtain landowner permission – The landowner must be willing to have the release site available for visitations and monitoring over several years. When getting permission for a site, be sure to secure the following:

1. Written permission from the landowner or land manager allowing use of the area as a release site.
2. Written agreement by the landowner allowing access to the site for monitoring and collection for a period of at least six years (three years for establishment and buildup and at least three years for collections).
3. Permission to put a permanent location marker at the site.

Preparing the Site

Preparing the release site involves the following activities:

- Some knapweed bioagents are so common and widespread that it is no longer necessary to redistribute them; for example, it is likely that the two *Urophora* flies (*U. affinis* and *U. quadrifasciata*) are already present. If so, it will not be necessary to release these flies at this site.
- Establish a permanent location marker: After selecting a site, choose a dense, uniform patch of knapweed in which to place a marker. Use brightly colored markers (wood or metal stake) to mark the exact location of the release site. The stake must be tall (about 4 ft. (1.2 m)) and clearly visible so that it can be easily found during future visits.
- Set up a photo point: A photo point (see "Establishing a Photo Point") is used to photographically record changes in knapweed infestation (decline or increase) over time following release of bioagents at a site.
- Draw a map: A map and written directions to the study site are essential for other people to locate the site. Note permanent roads, creeks, rivers, mile markers, etc. If possible, include a legal description or global positioning system (GPS) coordinates so that the site can be easily re-located.
- Baseline vegetation monitoring: In study sites where vegetation will be monitored, baseline data are used for comparing knapweed infestation measurements before and after releasing bioagents in the area. It is always a good idea to collect baseline vegetation data even at nursery or field release sites (see "Monitoring Vegetation").

2. Collecting Biocontrol Agents

Planning and timing of collection is critical. The type of bioagent and the knapweed species will dictate the best time in the season to collect. Ensure that all necessary collection supplies are on hand. The ability to accurately identify the bioagents is important.

Whether collecting larvae or adults, follow these general guidelines.

General Collection Guidelines

Quantity: The minimum needed to optimize establishment is 200 bioagents per site, but more is better.

Containers: Use "breathable" containers at all times. Breathable containers allow air flow to the insects and will not allow condensation. One of the best containers to use is a pint-sized, nonwaxed ice cream carton. These are breathable. Paper bags can work as temporary containers if care is taken to keep the bag from getting wet or squashed. Do not use plastic bags as containers because they are airtight and will not release moisture. Put a small wad of paper toweling in the container to absorb moisture and to give the insects crawling surface.

DO NOT USE PLASTIC BAGS AS CONTAINERS

Cooling: Keep bioagents shaded and cool at all times while collecting, sorting, counting and transporting. Bring a cooler with pre-frozen blue ice packs to the field. Secure an ice pack to the interior side of the cooler so that it does not roll around and crush the bioagents (for an example, see Fig. 66).

Sorting: Sorting is done after collecting to separate the insects from other organisms and debris, such as weed seeds, collected along with the insects. Empty the contents of the sweep net onto a tray and aspirate or hand-pick the insects out of the debris. For fast moving insects, keep them in the net and slowly open the top of the net and collect the insects as they attempt to escape. If the collected material is first chilled, the insects (especially beetles) move slower and are easier to collect.

Care: Exercise care in handling bioagents (see "Handling Biocontrol Agents"). Due to varying emergence times, difficulties that may be encountered when collecting bioagents are identified in Tables 9 and 10 (see also Appendix A: Troubleshooting Guide: When Things Go Wrong).

KEEP INSECTS COOL AND SHADED WHILE COLLECTING, SORTING, COUNTING OR TRANSPORTING THEM

Planning and Timing

Planning and timing of bioagent collection is critical. It involves knowing: (1) where, (2) when, (3) how, and (4) what to collect.

Where to collect

Identify the collection site. Determine whether bioagents are already established at the collection site by following the guidelines in "Selecting and Preparing a Release or Nursery Site". Whenever possible, choose a collection site closest to areas where the biogents will be redistributed. You may wish to consult with your county extension educator for an appropriate site.

When to collect

Determine the collection and release date(s) using the recommended timetables for knapweed seedhead feeders and root borers (Tables 11 and 12) as guidelines.

- Due to varying emergence time for individual insects, adult weevils can be seen at other than the optimal emergence periods given in Tables 11 and 12. However, for the greatest success in collecting adult bioagents, collect them during the peak emergence period.
- When sweeping for insects, the best time to collect is during the heat of the day (between 1:00 and 6:00 p.m.) because bioagents are more active at that time. Exceptions are *Sphenoptera* adults, which are nocturnal; the best time to collect them is early on warm evenings.
- Wait for a good day. Do not collect in the rain. Flying insects will not be around during a rain; crawling beetles will hide in protected niches and become more difficult to find. Excess moisture problems also occur easily when both the collected bioagents and collection containers get very wet.

How to collect

Choose a collection method to use on the desired life stage (larvae or adults) of the insects (see Tables 11 and 12). The five typical collection methods are as follows: sweep net, aspirator, handpicking, tapping (stick-bucket), and black light.

- **Sweep net:** A sweep net is made of cotton or muslin on a 10"-15" hoop attached to a 3' (0.9 m) long handle (Figs. 60 and 61). As its name implies, it is

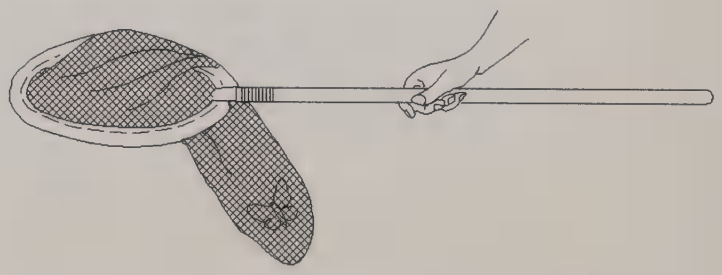


Figure 60. Sweep net used to collect knapweed biocontrol agents.

used to "sweep" insects off the knapweeds. The sweep net method is recommended for collecting adult beetles. It is relatively easy and efficient. It is best to sweep no more 50 times and then aspirating the insects, alternating between sweeping and aspirating. This reduces the potential harm that could result from knocking the bioagents around with debris or other insects inside the net. Flies are very delicate, thus collecting them with sweep nets can be harmful and even kill them. For this reason, sweep netting is not recommended for collecting seedhead flies. Sweep-netting delicate moths can also be harmful. Hence, when collecting adult moths, gently sweep the top half of the knapweed plants and confine them in the sweep net only for a short time (see Tables 11 and 12 for recommended times to use sweep nets).

Figure 61. Sweeping for knapweed insects.

Table 9. Level of difficulty in collecting knapweed seedhead feeders.

Insect	Life Stage	Method	Level of Difficulty[1]
Urophora affinis ***U. quadrifasciata***	Larva/pupa	Collect seedheads	●
	Adult	Sweep net	●●
Metzneria paucipunctella	Larva/pupa	Collect seedheads	●
Larinus minutus, ***L. obtusus***	Adult	Sweep net	●●
Bangasternus fausti	Adults	Sweep net or tapping	●●
Terellia virens	Larvae/pupae	Collect seedheads	●
	Adult	Sweep net	●●
Chaetorellia acrolophi	Larvae/pupae	Collect seedheads	●
	Adult	Sweep net	●●

Level of difficulty[1]: ● (easiest) to ●●●●● (most difficult)

Table 10. Level of difficulty in collecting knapweed root borers.

Insect	Life Stage	Method	Level of Difficulty [1]
Sphenoptera jugoslavica	Adult	Sweep net	●●●●
Cyphocleonus achates	Adult	Sweep net, hand pick	●●●
Agapeta zoegana	Adult	Aspirating Black light	●● ●●
	Larvae	Rearing from roots	●●●
Pterolonche inspersa	Adult	Sweep net	●●
Pelochrista medullana	Adult	Sweep net	●●

Level of difficulty[1]: ● (easiest) to ●●●●● (most difficult)

- **Aspirator:** Use an aspirator (Fig. 62) to suck the insects from the knapweed or the sweep net. It is sanitary (no unwanted or unknown material is inadvertently collected and assures the identity and quantity of the agent being released). Aspirating can be done in the field or indoors. When aspirating indoors, cool the insects to make them inactive and easier to aspirate. Seal and label the carton with the species, number of bioagents, collection site and date. Do not use aspirators for collecting adult moths because the scales from moth wings can break off during collection and get inhaled (by the collector).

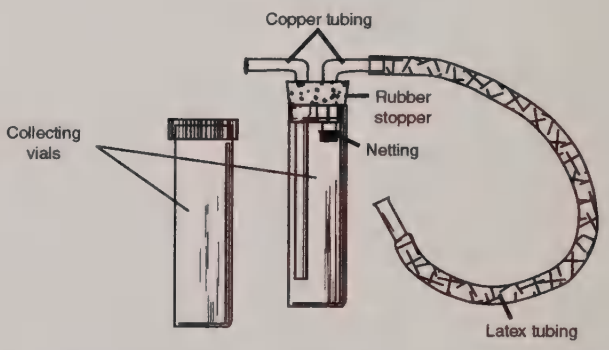


Figure 62. Aspirator used to collect knapweed biocontrol agents.

The use of a vacuum aspirator (Fig. 63) is another device for collecting insects. They are faster and more sanitary than a mouth aspirator. However, vacuums are cumbersome and not as easy to transport and use as a sweep net and mouth aspirator.

Figure 63. A hand-held vacuum aspirator (gasoline or battery powered).

- **Hand-picking:** Simply pick the insects from the knapweed by hand (with the aid of a pair of forceps, if desired). Hand-picking works best for stationary or slow-moving insects like the weevils *Bangasternus fausti* and *Cyphocleonus achates*.

- **Tapping:** If a sweep net is not available, tapping is the easiest collection method to use for collecting weevils. Using a stick (a badminton racquet works well), gently tap the knapweed stems into a bucket to remove the weevils. Separate the weevils further from unwanted debris and other plant materials, then place them in a breathable container. Use a funnel to aid in trapping the weevils into the bucket. To make a funnel, cut a plastic soda pop bottle in half, invert the small neck into the bottom and tape the two pieces together. Do not use funnels of this type for flies or moths.

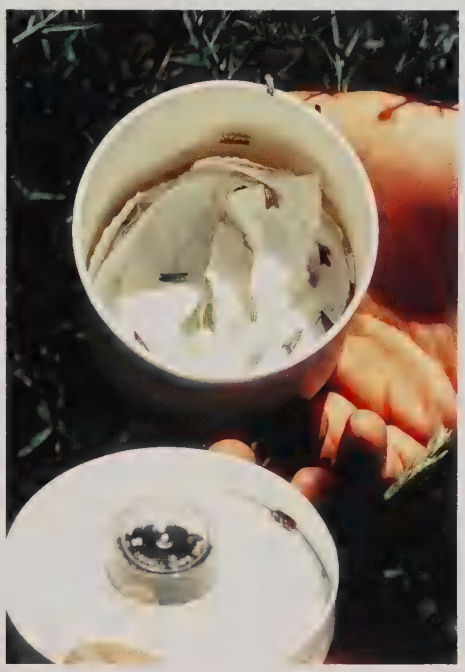


Figure 64. Carton lined with paper towel and containing adults of the knapweed seedhead moth *Metzneria paucipunctella*

- **Black light method:** Black ultraviolet (UV) lights attract moths at night. This method is used to monitor the nocturnal *Agapeta zoegana*. To use, suspend the black light from a post or set it up on top of a vehicle. Put a white sheet beneath the lights and on the ground to collect the moths that land on the sheet. Collect the moths into containers using a vacuum aspirator or hand-picking moths and place them in a container.

What to collect

The type of collection method to use will depend on whether you will be collecting adults or larvae (see Tables 11 and 12 for the appropriate life stage in which to collect bioagents).

Collecting adults

Of the three types of knapweed insects (flies, moths and beetles), the beetles are best collected as adults. Flies and moth adults are far more delicate than beetles and can be easily killed or harmed during collection. Carefully place adult moths in a tissue-filled container (Fig. 64).

The ideal weather for collecting insects is a sunny, warm day with a slight breeze.

It is best to collect bioagents when they are mating to ensure you are collecting both males and females and that eggs will be laid at the new site. It is important to have insects reproduce at the new site. If the insects are not mating and a collection is made too early, the collection may be mostly one sex and the new population may not establish at the release site. Collection made when insects are mating ensures that both sexes will be collected and released.

- **Beetles:** Adult beetles can be collected by hand or by sweep-netting. Hand-picking is also a suitable, though slower, method for collecting adult weevils. They are generally slow and, in the case of *Cyphocleonus* usually flightless.
- **Moths:** Collect adult moths with a sweep net. Note that the pheromone method can be used to collect male *Agapeta* moths.
- **Flies:** Sweeping adult seedhead flies is not recommended. Flies are fragile and can be damaged in the process of collecting, transporting and releasing them. Infested heads is the best way to collect and redistribute flies.

Collecting larvae

- **Beetles:** Seedhead weevil larvae are not generally collected. Beetle larvae in the root may not survive if the root is collected (dug out); thus adult beetles are usually collected (see also "Collecting Adults").
- **Moths:** The seedhead moth *M. paucipunctella*, can be easily and effectively collected as larvae in winter or early spring. Root-boring moths are best collected as adults although the moth *A. zoegana* can be collected as mature larvae in the root.
- **Flies:** Collect flies in the larval or pupal stage in March as they overwinter in the seedheads. Infested seedheads can be taken indoors to rear out adults or taken to the new site and left to complete their development under natural conditions.
    - **Direct placement:** The easiest method for releasing flies is to place bouquets of infested plants at the new site. Collect last year's plants in late winter, tie them into bouquets, take them to the new site and secure the bouquets to fence post or stake. Collect 50 seedheads from the bouquet, put them in a labeled paper bag or dry container and allow the insects to emerge indoors. This allows you to know which species you released at the new site.

- **Rearing:** Collect several hundred dry seedheads from last year's plants and put them in paper bags. Empty fly-infested seedheads from bag into a clear, breathable container such as a covered terrarium or petri dish. Leave the flies at room temperature. In 2-3 weeks, adult flies will begin to emerge from the seedheads. Another method is to put the seedheads in a rearing cage (Fig. 65). Collect the adult flies that emerge. Package the flies for shipment to a cooperator or bring them to a new site.

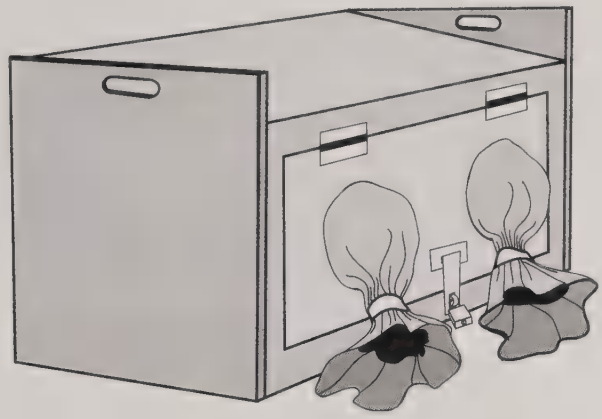


Figure 65. Insect rearing cage (also called a sleeve box).

Table 11. Recommended timetable for collecting knapweed seedhead feeders for redistribution.

	Flies			Beetles		Moths
	Urophora affinis	***Urophora quadrifasciata***	***Tyrellia virens*** ***Chaetorellia acolophi***	***Larinus minutus*** ***L. obtusus***	***Bangasternus fausti***	***Metzneria paucipunctella***
What to collect	Larvae or pupae in heads	Larvae or pupae in heads	Larvae or pupae in heads	Adults	Adults	Larvae or pupae in heads
Plant Growth stage	Rosette	Rosette	Botting	Flowering	Flowering - seed formation	Bolting-Bud
When to collect	Winter-Spring	Winter-Spring	Winter-Spring	Late June	Mid-July	Late winter
Method	Whole plant bouquets	Whole plant bouquets	Whole plant bouquets	Sweep net	Sweep net, tapping	Clip heads

Table 12. Recommended timetable for collecting knapweed root borers for redistribution.

	Beetles		Moths	
	Sphenoptera jugoslavica	***Cyphoclenous achates***	***Agapeta zoegana***	***Pterolonche inspersa*** and ***Pelochrista medullana***
What to collect	Adults	Adults	Adults	Larvae in the roots or adults
Growth stage	Flowering	Flowering	Late bud to flowering	Flowering
When to collect	Mid-July to mid-August during warm, calm, evenings	Warm, calm, cloudless days during peak emergence in August	Mid-July to late August	Mid-August (adults) late winter (larvae)
Method	Sweep nets	Hand pick from plants or sweep	Hand pick from plants black light and aspirator	Vacuum aspirator, sweep net or black light

3. Handling Biocontrol Agents

How the bioagents are handled after collection and transported to the release site can affect whether the bioagents will survive and multiply at the new site.

This section contains guidelines for transporting and shipping the bioagents.

Transporting the bioagents

How the bioagents are handled and transported can greatly impact whether they become established. It is best to redistribute the bioagents immediately after they are collected to prevent injury to the specimens, within 24 hours if possible.

Supplies Needed:

- Breathable container
- Masking tape
- Paper towel or styrofoam (for transporting)
- Cooler
- Blue ice pack
- Cardboard box (for shipping)

- **For immediate redistribution** - Store the insects in a breathable container with fresh knapweed foliage or 1 inch of slightly damp sponge (must not contain dyes or phosphates) or paper towel.
- **For later redistribution** - Store the insects in a refrigerator. Bioagents can last up to three days in a refrigerator; however, only one day of refrigerated storage is recommended. For storage longer than one day, follow the guidelines for keeping bioagents alive during transportation, shipping or storage.
- **Seal and label the container** - Seal with tape and label the container with the name of the bioagent, the quantity collected, and collection date. Tape a blue ice pack to the bottom of the cooler to avoid physical damage. Put a barrier (e.g., newspaper) between the ice pack and the biogents to protect the bioagents from excess moisture or cold.

DO NOT ALLOW CONTAINERS TO TOUCH THE BLUE ICE PACK

Shipping the bioagents

To ship bioagents over a long distance, plan the route and timing of shipments to prevent undue delays and stress on the bioagents. Ship the agents by overnight courier and instruct your cooperator to release them within 24 to 48 hours of receipt. Overall, observe the following guidelines:

- Know the regulations – Observe appropriate rules, restrictions and regulations pertaining to shipping bioagents to a cooperator or moving bioagents out of

the county or state. For the current regulations, contact your local weed district, cooperative extension agent, the state Department of Agriculture, or the USDA Animal and Plant Health Inspection Service (APHIS).

- Prepare the bioagents - Sort the bioagents from all other unwanted material to avoid contamination at the receiving site.
- Shipping containers - Put bioagents in containers with enough space to allow the insects to move about within the container.
  - Line the container with a crawling surface for the insects (such as a wadded paper towel).
  - Provide the insects with food and adequate ventilation while in transit. If using knapweed plant material as a food source, do not include roots and seeds. Place tissue in container with moths and flies to give them a surface and crevices to rest in. This method is not recommended for moths or flies as the plant material can shift in transit and damage the insects. Fresh knapweed foliage will provide insects with sufficient water. Do not put water in the containers.
  - Tape lids on the containers and make sure that the biogents do not get caught on the sticky part of the tape.
  - Prevent damage to the bioagents by packing the shipping container with care. Keep the bioagents cool until they can be released. Tape the blue ice packs to the inner side of the chest and pack with a layer of paper to absorb condensation (Fig. 66).

Common Mistakes

- **Excess heat** - Do not expose biogents to direct sunlight
- **Excess moisture** - remove spilled or excess water in the container
- **Lack of air** - Provide adequate ventilation; use only breathable containers.

Figure 66. Shipping box containing agents in cartons, styrofoam to prevent shifting, and blue ice packs.

Summary: Care of the Bioagents

- Provide a crawling surface for the bioagents, such as knapweed leaves and stems or tissue.
- Avoid physical damage to the bioagent by taping down potentially harmful objects, such as blue ice packs.
- Ensure that predators (i.e. spiders) are not trapped with the bioagent in the container by sorting bioagents before packaging them.

- Provide container with adequate ventilation.
- Do not expose bioagents to excessive heat.
- If release or shipping is not immediate, store the bioagents in refrigerators no colder than 40º-50º F (4ºC) for a no longer than 2 days or keep them in an ice chest until the bioagents are ready to be shipped or transported. Longer storage decreases the bioagents' chance for survival at the new site.

4. Releasing Biocontrol Agents

Timing of the release will determine whether the bioagents will survive and flourish at the new site (Tables 11, 12 and 13). Follow these steps for releasing bioagents:

1. Place the permanent location marker - Release the insects at the location marker. This location will be later used in monitoring activities.
2. Make the release - Consult Table 13 to determine the appropriate method to use for releasing each insect.
3. Take pictures - Take a series of photographs to record the release. A photo point will record the change in the site over time. For further information, see "Establishing a Photo Point".
4. Collect baseline vegetation data - Choose a monitoring method listed in Tables 14 and 15. Establish baseline data at the time of the release. Use the same monitoring method every year.
5. Fill out and submit a release form - Complete the Biological Control Release Form (see Appendix B for a sample of the Bioagent Release Form). Submit the form to your county extension educator, university or state department of agriculture. Keep a copy for your records.

Timing the release

Release bioagents (Fig. 67) at the appropriate growth stage of the knapweed (review "Selecting and Preparing a Study Site") or check with your county extension agent or county weed supervisor. If most knapweed buds are beyond the recommended stage, it is too late to release at that site.

Do not wait for good weather. If you must release in the rain, provide shelter for the bioagents until they can disperse on their own. One way to do this is to place a cardboard box on its side, place the container in the box and open the lid. The bioagents will disperse when weather conditions improve.

Table 13. Appropriate release method for each bioagent.

Agent	Release Method
Urophora affinis	Tie bouquets of infested seedheads to a fence post
Urophora quadrifasciata	Tie bouquets of infested seedheads to a fence post
Terrelia virens	Release 200 adults within 24 hrs of emergence
Chaetorellia acrolophi	Release 200 adults within 24 hrs of emergence
Metzneria paucipunctella	Release 200 adults OR place infested seedhead
Larinus minutus	Release 200 adults
Larinus obtusus	Release 200 adults
Bangasternus fausti	Release 200 adults
Agapeta zoegana	Release 100-200 adults
Pterolonche inspersa	Release 200 adults
Pelochrista medullana	Release 200 adults
Cyphocleonus achates	Release 100-200 adults, depending on difficulty of collection
Sphenoptera jugoslavica	Release 100-200 adults, depending on difficulty of collection

The two methods of releasing bioagents are as follows:

- **Open-field release:** When releasing adult weevils or flies, place the bioagents on the ground within a 3-ft. radius of the permanent location marker under knapweed plants where they can continue to mate and disperse on their own.

- **Caged release:** An alternative to open-field release is to put the insects in a release cage or tent (Fig. 68). The bottomless tent, placed over a patch of knapweed, is very useful in keeping flying insects together while giving them "natural" conditions. Another simpler release cage is constructed from plastic milk jugs (Fig. 69) used for seedhead flies and moths. The cover over the jug keeps the seedheads dry; newly emerged adults can escape through the hole under the handle, and seeds are not released into the environment (the jug, with seeds, can be removed later).

Figure 67. Releasing knapweed bioagents on spotted knapweed.

Retain voucher specimens

Retain 5-10 bioagents (dead or alive). Put the bioagents in a small vial with 70% ethyl alcohol for a voucher specimen. On a piece of white paper, in pencil **(always use a pencil because alcohol will dissolve and bleed ink from pens and markers)**, write the name of the bioagent, source of the bioagent, the date of release, person or agency releasing, and release site. Put inside the vial. This identifies the bioagent released for future reference. Save several specimens for in-house records. Send the voucher specimens to your county extension educator or weed biocontrol expert.

Figure 68. Screen tent cage in which to release and contain knapweed bioagents.

Frequency of release

More than one release per season may be appropriate if a prior release fails. If more releases are to be made, sites can be a short distance apart. Root insects are usually released 2-3 times per site as they are slower and more difficult to collect.

Releasing multiple bioagents

It is expected that bioagent populations will overlap and eventually sort themselves out naturally depending on the habitat, population density, and weed levels. As a rule, however, separate the species by at least 100 m to allow your insect to establish without being impeded by another species.

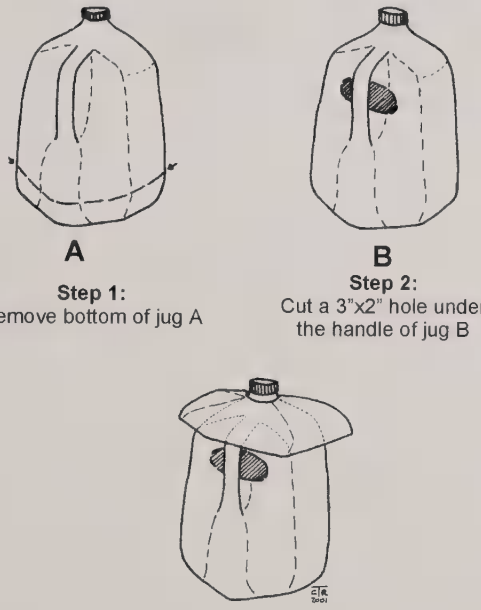


Figure 69. An example of a milk jug release cage for knapweed bioagents.

Suggestions for optimal establishment

- A release of 200 bioagents is optimal.
- Releasing in the early morning hours between 6:00 and 10:00 a.m. or in the cooler evening hours between 7:00 and 10:00 p.m. is recommended. Bioagents are less likely to fly away immediately when released in cool temperatures.
- Avoid releasing bioagents during rainy or very hot weather for optimal establishment. However, sometimes it may be necessary to release in the rain.
- Releasing weevil bioagents at the bottom of a hill may encourage them to follow the phenological stages of the knapweed uphill, which may possibly increase the rate of spread; however, there is no data to support this observation.
- Common sense and care is a major factor in the survival and establishment of the insects.

Questions to Ask

- Are the bioagents already present at the site?
- Did the bioagents successfully establish following release?
- Are the bioagents found in high enough density to be collected and distributed?
- How far have bioagents dispersed from the initial release sites?
- Are the bioagents causing visible damage to the target weed?
- Are changes occurring within the plant community?

Answers to these questions will allow land managers to do the following:

- Determine the success of biological control efforts for target weed populations.
- Determine if a supplemental release is needed.
- Establish that biocontrol agents are impacting the target weed.
- Document changes in the plant community.

5. Monitoring Biocontrol Agents

Monitoring of the release site is conducted to: (1) ensure that the bioagents have established, (2) determine of the bioagents have spread from the release site, and (3) assess the impact of the bioagents on the targeted knapweeds. Monitoring requires little effort but provides useful information. It can be as simple as asking the right questions and getting the correct answers (see *Questions to Ask* box above).

When to begin monitoring

- Some bioagents may be detected as early as 1 year following release.
- Some bioagents take 2 to 3 years to be detectable. Thus, if no bioagents are detected a year after the release, it does not mean that the insects failed to establish. Revisit the site for three years. If no evidence of insects is seen, either choose another site or make additional releases (see Appendix A: Troubleshooting Guide for reasons on why agents fail to establish). Consult with your county extension educator or local biocontrol of weeds expert.

Monitoring Bioagents

There are a number of methods used to monitor insects. These are:

- Visual observations along a transect to count adults.

Table 14. Recommended timetable for monitoring knapweed seedhead feeders.

	Spring		Summer		Fall		Winter	
	Early	Late	Early	Late	Early	Late	Early	Late
Urophora affinis	Dissect year old seedheads for larvae and pupae		Collect adults by sweeping				Dissect year-old seedheads for larvae and pupae	
U. quadrifasciata	Dissect year old seedheads for larvae and pupae		Collect adults by sweeping				Dissect year-old seedheads for larvae and pupae	
Terellia virens	Dissect year old seedheads for larvae and pupae		Collect adults by sweeping				Dissect year-old seedheads for larvae and pupae	
Chaetorellia acrolophi	Dissect year old seedheads for larvae and pupae		Collect adults by sweeping				Dissect year-old seedheads for larvae and pupae	
Metzneria paucipunctella	Dissect year old seedheads for larvae and pupae		Collect adults by sweeping				Dissect year-old seedheads for larvae and pupae	
Bangasternus fausti		Sweep mating adults			Count exit holes in seedheads (see Fig. 71)			
Larinus minutus		Sweep mating adults			Count exit holes in seedheads (see Fig. 71)			
L. obtusus		Sweep mating adults			Count exit holes in seedheads (see Fig. 71)			

- Sweep net sampling to count adults.
- Using black light to capture and count nocturnal adult moths.
- Use of pheromone traps to capture adult male *Agapeta zoegana* moths.
- Dissecting roots and seedheads to observe larvae.

Monitoring Methods

It is typically necessary to collect bioagents in order to monitor their population and activity. The collection methods described in "Collecting Biocontrol Agents" work just as well in monitoring insects. The monitoring method you choose depends on:

- Life stage of the insect
- Amount of time available
- Expertise of the observer
- Availability of equipment
- Monitoring objective

Table 15. Recommended timetable for monitoring knapweed root borers.

	Spring		Summer		Fall		Winter	
	Early	Late	Early	Late	Early	Late	Early	Late
Agapeta zoegana	Dissect roots for larvae			Trap males in pheromone traps				
				Aspirate adults from plants, Visual counts, Black light				
Pelochrista medullana	Dissect roots for larvae or pupae		Sweep adults Black light					
Pterlonche inspersa	Dissect roots for larvae or pupae		Sweep adults Black light		Sweep or hand-pick adults			
Cyphocleonus achates	Dissect roots for larvae or pupae			Sweep Adults Visual Counts				Dissect roots for larvae
Sphenoptera jugoslavica	Dissect roots for larvae or pupae			Sweep adults Visual counts				Dissect roots for larvae

For example, to determine if bioagents are merely established at the release site, observing any life stage is adequate. To determine the density of insects at the release site (i.e., number of insects per root), more detailed and intensive monitoring is needed. Likewise, if you want to know how far the bioagents have spread from the release site, a more systematic monitoring is needed.

Additional Monitoring Methods

Insect monitoring uses the same methods used to collect bioagents for release (see "Collecting Biocontrol Agents"). Additional monitoring methods including pheromone trapping, manual counting, and dissecting roots, are discussed below.

Figure 70. Pheromone trap used to collect adult male *Agapeta zoegana* moths.

- Pheromone Trap Method -Pheromones are chemical attractants (odor) exuded by insects to attract the opposite sex. They are highly specific to one species of insect. Pheromones are used in many areas of insect pest management. The pheromone is artificially synthesized, packaged, placed in a trap and set out in the field. In the biocontrol of knapweed, this method is currently only available for the knapweed root moth, *Agapeta zoegana*. The pheromone trap, called a Delta trap (Fig. 70), is used to attract male moths. It is considered by some to be the best monitoring method for this insect.
- Manual counting of adults - This is an easy, fast and inexpensive way to monitor insects. Using six to ten, 60-feet (20 m) long transects radiating away from the permanent location marker at the release site, count the number of adult insects you see on or near the plants in a 3-5 foot (0.9-1.6 m) circle every 20 feet (6.6 m) along the transect.
- Dissecting roots for bioagent larvae - This method is used to count larvae found in the knapweed roots. Using a 30-foot (9.8) transect, dig a root (no smaller than ¾ inch (1.9 cm) diameter at the crown) every 3 feet (0.9 m). Roots can be examined on-site or taken back to the office or laboratory for dissection later. Cut the root longitudinally to expose the larva(e).

A Method for Sampling Plants to Evaluate Feeding Damage

Collect six plants along each of four lines in four cardinal directions (N, S, E, W) from the permanent location marker, for a total of 24 plants. Heads can be dissected indoors to see if they contain bioagent larvae or pupae. When sampling roots, dig one root at a time and slice it lengthwise to expose the center.

- Label collection bags with site name, date and transect, and take the collected plants indoors for detailed examination later.
- Count and record the total number of buds, flowers and mature seedheads collected from each plant. Dissect each bud carefully. Seedheads with weevils can be examined for damage by counting the number of exit holes on the plants (Fig. 71).

Figure 71. Emergence hole in spotted knapweed seedhead created by new adult weevils exiting the seedhead.

Monitoring Vegetation

Vegetation monitoring is conducted to describe and measure changes in the knapweed population following the release of bioagents. It consists of taking multiple measurements of a variable, such as plant height, density or number of seedheads. Analysis is performed to determine if changes in the weed infestation have occurred. The type of vegetation monitoring to use depends on the type of site (e.g., study or nursery site), availability of resources, and your monitoring objective.

Monitoring objectives

Develop a plan for collecting data based on the monitoring objectives. Use the "Monitoring Plan Checklist" in Appendix C to determine the objective or purpose of monitoring. The two types of monitoring objectives are low intensity and quantitative:

Low intensity monitoring objectives

Two examples of low intensity monitoring objectives are:

- Determining the density and distribution classes of knapweed at the release site over 2-year intervals. Distribution classes are seedlings, rosettes, bolted and mature.
- Creating a photo record beginning at the time of bioagent release and at 2-year intervals thereafter (called before-and-after photos). Trends and changes in the knapweed infestation and the plant community can be visually assessed with photographs.

Quantitative monitoring objectives

The objective of quantitative monitoring is to record and measure changes in the knapweed population that can be attributed to the release of bioagents. This level of monitoring is amenable to more detailed statistical analysis and produces more precise information about the vegetation.

Types of Monitoring

Monitoring can be as general as before-and-after photos (qualitative) and as specific and intensive as conducting field studies for accurate and detailed assessment (quantitative).

1. **Qualitative monitoring** - This method uses descriptive elements about knapweed at the management site. It includes such general recording of presence or absence of bioagents, estimates of density, age and distribution classes, infestation mapping, and permanent photo points. Qualitative monitoring is quick and inexpensive, and provides some insight on the status or change of the knapweed population. However, it is less repeatable than other methods and its descriptive nature does not allow for detailed statistical analysis. In addition, interpretations derived from this type of monitoring are often subjective.

2. **Quantitative monitoring** - This method involves the quantification of weed or site variables (Fig. 72). Quantitative monitoring can be simple or detailed. An example of simple quantitative monitoring is a single permanent quadrat or transect established at a study site used to count the number of flowering knapweed plants. Detailed quantitative monitoring involves the sampling of population/site variables. The data are then analyzed and generally give a more precise information on plant population or community changes. Other differences include:

Figure72. Measuring knapweed height at a quantitative monitoring site.

Simple quantitative monitoring

- Measurement (e.g., plant height) can be used as an index to track changes in a population
- Data can be compared qualitatively over time
- May trigger more intensive monitoring.

Detailed quantitative monitoring

- Sampling is more detailed (e.g., plant height, rosette diameter, number and size of seedheads, percent cover, species diversity).
- Population is sampled over time to determine changes and trends.
- Repeatable and allows for broad statistical analysis.
- Takes more time to plan and implement, making it more expensive. It may also require specialized skills and training.

Qualitative monitoring

To conduct qualitative monitoring, follow these steps:

Choose location to monitor - Begin monitoring where the bioagents were first released since this is where the highest density agents is likely to occur and therefore where changes to the knapweed are more likely to be detected.

Visual estimates - Record visual estimates of canopy cover, density, and distribution classes of knapweed within the monitoring area. Personnel may have to be trained in estimating general vegetation attributes.

Documentation - Fill out a site documentation form (see Appendix C: Biocontrol Monitoring Report for an example) and check the appropriate boxes that best describe the knapweed and other life forms at the release site.

Photo point - Establish a permanent photo point in the monitoring area. The photo point is an area where estimated cover and/or density classes of the knapweed can be recorded. Be sure to label the photo point.

Monitoring schedule - Schedule monitoring activities at the same time each year to be consistent and compare year-to-year variation. Qualitative monitoring is not used to track small, yearly changes in knapweed population attributes,

Quantitative monitoring

Quantitative monitoring is more difficult than qualitative monitoring in that it requires careful planning, selection of sites, and appropriate use of monitoring methods.

Planning - This step involves knowing what and how much data to collect before starting. Consult an experienced field technician, researcher or statistician for guidance on the design of your monitoring plan.

Choose location to monitor - As in qualitative monitoring, choose a site where biogents were first released. If a post or stake has been placed at the release site, determine and record its bearing and distance from the site post to the monitoring plot.

Choose monitoring method - Choice of a qualitative monitoring method depends on the amount of time available to conduct the work and the monitoring objectives. The two methods of quantitative monitoring are: (a) macroplots and (b) permanent transects.

Macroplot

The purpose of the macroplot is to define a large area (e.g., 4 acres (1.6 hectares) within which randomly placed small quadrats (1 sq. yd or 1 sq. m) are used to sample vegetation (see Appendix G: Macroplot Design for Measuring Density for an example of a macroplot layout). The macroplot allows for sampling over a large area but can cause considerable trampling at the site during sampling.

Transect

A transect is a straight line measured on the ground along which vegetation is sampled. Transect lines can be as long as 300 ft. (100 m) or as short as 30 ft. (10 m). Vegetation along the transect is sampled or measured using a quadrat placed at regular intervals along the transect (i.e., every 10 ft. (3.2 m). While transects are a more systematic method of sampling vegetation, the location of the transect can be random. Transects are faster and easier to set up and use than macroplots.

Supplies Needed

- camera (35 mm or digital)
- color film (if 35 mm)
- notebook and forms
- metal or wooden stake for camera point
- bright-colored spray paint
- Previous year's photo

Measuring Vegetation

- Count the number of seedlings, rosettes and flowering knapweed plants within the quadrat.
- Estimate the percent cover of plants species or general life forms.
- Repeat for each quadrat.
- Monitor at the same time of year at the predetermined intervals established in the monitoring plan.
- Select a new set of coordinates at random each time the macroplot is sampled.

Establishing a Photo Point

Photographs of the release site are a valuable assessment tool. Visual evidence of vegetation change over time is derived from comparing pictures of the same site taken from the same location, at the same time of year, with the same horizon, and taken over a period of years. Records consisting of photographs are a qualitative form of monitoring and can be used in conjunction with more intensive quantitative monitoring techniques (see "Monitoring Vegetation").

- **Take baseline photographs at the time of the release** - Choose the time of year to take the first set of pictures; flowering stages are ideal because of the contrasts with the surrounding vegetation. Once a year is sufficient but it is good practice to frequently take pictures of the site.

- **Locate a photo point** - The location of the photo point is determined at the time of establishing the release site. Note and document the location of the photo point marker in case of need to relocate it later. Take photographs of the site from the camera point toward the permanent location marker.
- **Close-up pictures** - Close up pictures are useful to show the amount of ground covered by vegetation and litter. A square frame measuring 3′ x 3′ is recommended. Frames can be made of PVC pipe, steel rods, rebar, etc. Drive brightly painted angle iron stakes into the corners to permanently establish the plot. Repaint the stakes each time photos are taken. Put a plot identification label on the ground next to the frame. The camera point should be on the north side of the photo, so that pictures can be taken at any time of the day without a shadow.
- **General view pictures** - General view pictures give a broad view of the release site and the surrounding landscape.
    - Establish the point approximately 100 feet from the permanent location marker.
    - Choose an angle that will best show changes in the knapweed infestation over time.
    - Include a photo identification label, a general view of the site, some sky, and a reference point in the foreground (fence post, shrub, or person), and a distinct landmark on the skyline.
    - Use pictures taken the previous year as reference for the following year's photos.

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Glossary

achene A small, one-seeded fruit that does not split at maturity.

alternate Leaves that are arranged singly along a stem; one leaf or bud at each node on alternate sides of the stem.

aspirator An apparatus used to suck insects into a container. Can be as simple as in mouth-aspirator, or mechanical as in a gasoline- or battery-powered vacuum aspirator.

basal At the base of a plant or plant part.

biennial A plant which lives two years.

biological control The intentional use of a weed's natural enemies for control purposes. Also referred to as biocontrol.

bolting Plant stage at which the flower stalk begins to grow.

bract A small, leaf-like structure below a flower.

capitulum (pl. capitula) Seedhead of plants in the sunflower family.

coordinate Any of a set of numbers used to specify a point on a line.

cotyledon First leaf-like structures that appear after germination; seed leaves.

density Number of individuals per unit area.

dissemination Dispersal. Can be applied to seeds or insects.

elytron (pl. elytra) Hardened front wing of a beetle.

emergence Act of adult insect leaving the pupal exoskeleton.

exoskeleton External skeleton of the body of an insect.

floret One of the small, closely clustered flowers forming the head of a composite flower in the sunflower family.

frass Plant fragments, usually mixed with excrement, deposited by feeding insects.

gall An abnormal growth on a plant, usually induced by an insect that lives within the gall.

grub A soft, thick-bodied, C-shaped beetle larva.

head A group of flowers borne tightly together.

host specificity The highly-evolved, often obligatory association between an insect and its host (i.e., weed).

inflorescence The flowering part of a plant.

instar The phase of an insect's development between molts.

involucre A circle of bracts under an inflorescence.

larva (pl. larvae) Immature insect stage between the egg and pupa.

lobed A leaf with shallow or deep, rounded segments, as in a knapweed rosette leaf.

metabolic sink Site of the plant that receives photosynthate (food) produced by the plant, diverting the resource from the plant's normal use.

molting Process of insect development that involves shedding its exoskeleton and producing an exoskeleton for the next instar.

mottled Surface having colored spots or blotches.

organdy A fine transparent cloth.

oviposit To lay or deposit eggs.

ovary The part of the flower that contains the ovules or seeds.

pappus A tuft of hairs, scales or bristles at the tip of an achene in flowers of the sunflower family.

perennial A plant that lives more than two years.

pheromone A substance given off by an insect used to communicate with other insects of the same species.

proleg A fleshy, unsegmented, abdominal walking appendage of some insect larvae, common among caterpillars.

pubescence Hairs covering a leaf, stem, or flower.

pupa (pl. pupae) (v. pupate) Non-feeding, inactive stage between larvae and adult in insects.

puparium The hardened, thickened skin of a mature larva within which the pupa and adult are formed.

quadrat A specific area used to sample vegetation (e.g., 1 square meter).

qualitative Measurement of descriptive elements (e.g., age class, distribution)

quantitative Measurement of quantity - number or amount (e.g., seeds per capitula).

receptacle Part of the stem to which the flower is attached.

rosette A compact, circular, normally basal cluster of leaves.

senescence Final stage in a plant's lifecycle.

snout 'Nose' of a weevil. The elongate head of a weevil with mouth parts at the apex.

spine A stiff, pointed plant part.

synchrony Occurring at the same time.

thorax Body region of an insect behind the head and abdomen, bearing the legs and wings.

transect A straight line of varying length along which plants are periodically sampled individually or in quadrants.

univoltine Produce only one generation per year.

variable A quantity that can have more than one of a set of values (e.g., plant height).

weevil A type of plant-eating beetle; the adult has a snout, and the larva is a C-shaped grub (aka snout beetle).

x-axis Horizontal axis or line in a coordinate system.

y-axis Vertical axis or line in a coordinate system.

Appendix A:

Troubleshooting Guide:

When Things Go Wrong

TROUBLESHOOTING GUIDE: WHEN THINGS GO WRONG

This guide is intended to assist those who encounter problems when establishing a biological control program. It identifies the probable cause of a typical problem and

Problem	Probable Cause	Solution
Bioagents unhealthy	Physical damage to agents	Prevent containers from colliding; use crush-proof containers
	Drowning	Do not put water in containers. Prevent accumulation of excess moisture in the collection container
	Excess or prolonged heat or cold	Keep container cool at all times; use coolers and blue ice packs; avoid exposure to direct sunlight while in transit
	Starvation	Put knapweed foliage (no flowers, seeds, or roots) in container
	Redistribution time	Transport or ship agents immediately after collection
		Release agents at new site immediately upon arrival or receipt of agent
	Parasitism and/or disease	Check source of agents Ensure insect population is disease-free when collecting or receiving shipment
Number of eggs low	Agents past reproductive stage	Collect at times of peak activity (ie: insects are reproducing)
	Sex ratio: not enough males or females	Observe mating among bioagents before collecting; males often emerge earlier than females
	Synchrony	Agents not synchronized with knapweed growth stage; bioagents require knapweed to be at specific growth stage for optimal oviposition

Troubleshooting Guide (continued from previous page)

Problem	Probable Cause	Solution
Few bioagents collected	Wrong method used	Refer to Tables 11 and 12 for recommended collection time and technique
	Collection done at wrong time	Refer to Tables 11 and 12 for recommended collection time and technique
	Collection technique	Bioagents can be killed during sweeping or aspirating Use vacuum aspirator if aspirating by mouth is not working Practice sweeping
	Conditions at time of collection wrong	Refer to "Collecting Biocontrol Agents" and "Monitoring Biocontrol Agents" for guidelines on desirable weather conditions
Agents not found after release	Site is unsuitable Climate is unsuitable Elevation Aspect	Refer to "Collecting Biocontrol Agents"
	Site too small	Select a larger site with a dense, uniform stand of knapweed
	Pesticide used in area	Select pesticide-free site
Cannot locate release site	Permanent location marker not obvious	Use bright-colored wooden, metal or plastic stake
	Map poorly or incorrectly drawn	Check map; redraw with more detail or add landmarks

Appendix B:

Sample

Biocontrol Agent Release Form

BIOLOGICAL CONTROL RELEASE FORM

AGENT RELEASE

Released By:_____ **Release Date:**___/___/___ (mm dd yy) **County:** _____ **State:**_____

Agent:_____ **# Released:**_____ **Target Weed:**_____

Source of Agents:_____ **Date Collected:**___/___/___ (mm dd yy)

Life Stage (circle)**:** Larvae Pupae Adults Eggs Other (specify) _____

Land Ownership (circle)**:** Private County State USFS BLM COE BOR BIA/Tribe TNC Other (specify) _____

Legal: T_____ R_____ Sec_____ Q_____ QQ_____ (OR) **Lat:** Deg____Min____Sec_____ **Long**: Deg___Min_____Sec_____

ENVIRONMENT

Temperature (°F):_____ **Wind:** Calm, Light, Moderate, Strong, Gusty **Wind Direction:** N S E W

Weather (circle)**:** Clear, Ptly Cloudy, Cloudy, Rain, Snow **Release Time** (military):_____

Site Aspect (circle)**:** N, NE, E, SE, S, SW, W, NW **Elevation:**_____

Site Slope: Flat (0-10%) _____ Gentle (10-30%) _____ Moderate (30-60%) _____ Steep (>60%) _____

Topographic Position (circle)**:** Valley Bottom Terrace Toe Slope Low/Mid/Upper Slope Crest

Disturbance: (check all that apply, circle most prevalent) Cultivation ___ Fire ___ Flood ___ Grazing ___ Logging ____ Roads____ Mining ____ Recreation ____

Directions to Site:_____

_____

SITE CHARACTERISTICS

Site Name:_____ **Size of Infestation** (acres)**:**_____ **Weed Cover %:**_____

Weed Height:_____ **Weed Density** (# per meter sq.)**:**_____ **Dominant Plant:**_____

Distribution of Weed: Isolated_____ Scattered_____ Sc-Patchy_____ Patchy_____ Continuous_____ Linear_____

Phenology: Seedling % _____ Rosette %____ Bolt %_____ Bud %_____ Flowering %_____ Fruit %____ Dormant %_____

Vegetation Type:

Annual Grassland _____
Perennial Grassland _____
Shrubland/Steppe _____
Dry Conifer _____
Mixed Conifer _____
Dry Meadow _____
Moist Meadow _____

% Tree Cover _____
% Shrub Cover _____
% Forb Cover _____
% Grass Cover _____
% Litter Cover _____
% Bare Ground Cover _____
% Rock Cover _____

Soil Texture: (check) Sand___ Silt___ Clay___ Gravel___ Loam___

Comments (continue on reverse if necessary):

Appendix C:

Monitoring Plan Checklist

MONITORING PLAN CHECKLIST

The following is a list of questions to be answered and documented prior to collecting data. Use the checklist as an outline for a monitoring plan.

What is the management objective of the biocontrol release site? _____

What is the monitoring objective of the biocontrol release site? _____

What will be measured? _____

What equipment and supplies are needed? _____

What training is needed? _____

What is the cost of monitoring? _____

What is the interval between monitoring? _____

Appendix D:

Biocontrol Monitoring Report

BIOCONTROL MONITORING REPORT

Release Site Location:			**Date**:	
Site name				
State			County	
Nearest town			Road/mile marker	
Legal Description	Township	Range	Sec	
GPS	Latitude (Deg) (Min) (Sec)		Longitude (Deg) (Min) (Sec)	
TARGET WEED			Est. weed density _____/sq.m	
Plant cover (Estimate %) target weed_____ forbs (not including target)_____ grasses_____ shrubs_____ trees_____ litter_____ bare ground_____				
BIOCONTROL AGENT				
Agent Released	Species Released: _____		Release date:	
MONITORING INFORMATION				
Sampling date:			Sampling time:	
Source of agents	Collection date		Collection location T_____R_____S___	
	Collected by		Lat Long	
No. released:	Weather conditions:			
Agent stage present ☐ egg ☐ larvae ☐ pupa ☐ adult				
Weed stage present ☐ seedling ☐ rosette ☐ bolting ☐ flowering				
Other agents present (list):				

Directions to release site:

Appendix E:

Qualitative Monitoring Form

Knapweed
Qualitative Monitoring Form

Name: _____ **Date:**_____ **Time:** _____ **am/pm** _____

Location: _____ **Site #:** _____

Insect:_____ **Year of release:** _____

Cover Class by Plant Type						
	0%	1-5%	6-20%	21-45%	46-70%	71-100%
Knapweed						
Annual Grasses						
Perennial Grasses						
Forbs						
Shrubs						
Trees						

Dominant Plants on Site:
Other Noxious Weeds:

Knapweed density class (✓check one)			
Flowering plants/meter sq)		Knapweed distribution	
0		Isolated	
1-25		Scattered	
26-50		Scattered-Patchy	
50-75		Patchy	
>75		Continuous	

Knapweed phenology Class at time of monitoring	
Knapweed Stage	Estimated percent
Seedling	
Rosette	
Bolting	
Flowering	
Senescent	

Comments/Observations _____

_____

_____

_____

_____

_____

_____

Appendix F:

Quadrat Density and Cover Data Form

Quadrat Density and Cover Data Form

Date:	Examiners:	Site Name:
Location:		T. : R. : Sec. : Q Sec. : Q Sec
Description:		Lat. Long.

Plot No.	1		2		3		4		5		6		7		8	
Species	Density	% Cover	Density	% Cover	Density	% Cover	Density	% Cover	Density	% Cover	Density	% Cover	Density	% Cover	Density	% Cover

Plot No.	9		10		11		12		13		14		15		16	
Species	Density	% Cover	Density	% Cover	Density	% Cover	Density	% Cover	Density	% Cover	Density	% Cover	Density	% Cover	Density	% Cover

Appendix G:

Macroplot Design for Measuring Density

Example of Macroplot Design for Measuring Density

Designed for a 1 x 2 ft quadrat in a 22 x 22 ft. macroplot

The X-axis is in 2 ft. increments; the Y-axis is in 1 ft. increments.

Y-axis												
22											XX9	
21							XX5					
20												
19			XX2									
18												
17												
16												
15				XX3								
14												
13												
12	XX1									XX8		
11						XX4						
10												
9									XX7			
8												
7												
6												
5												
4								XX6				
3												
2												
1												XX10
	1	2	3	4	5	6	7	8	9	10	11	12
	0	2 ft	4 ft	6 ft	8 ft	10 ft	12 ft	14 ft	16 ft	18 ft	20 ft	22 ft

---------- X-axis ----------

- each square (cell) is a quadrat measuring 2 ft x 1 ft.
- vertical and horizontal numbers are the coordinates of the X and Y axes respectively
- xx = randomly selected quadrat to be sampled

Examples: XX1 = coordinate (1,12); XX2 = coordinate (3,19); XX3 = coordinate (4,15);
XX4 = coordinate (6,11); XX5 = coordinate (7,21); XX6 = coordinate (8,4);
XX7 = coordinate (9,9); XX8 = coordinate (10,12); XX9 = coordinate (11,22);
XX10 = coordinate (12,1)

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Sources of Figures

Cover photo	University of Idaho, C. Roché and Nez Perce Biocontrol Center
Figures 1, 7, 10, 13, 16, 20, 21a, 23, 26, 34, 36, 39, 40, 57-59, 61, 64, 66, 67, 72	University of Idaho
Figures 2, 27, 28, 43	L. Wilson, University of Idaho
Figures 3a, 4-6, 8a, 9, 11a, 11b, 12, 14a, 14b, 15, 17-19, 21b, 22, 32a, 32b, 47, 69	C. Roché, Medford, OR
Figure 3b	http://pc65.frontier.osrhe.edu
Figures 8b, 42, 46, 48, 49, 56, 68, 71	Nez Perce BioControl Center, Lapwai, ID
Figures 7, 10, 13, 20, 23	USDA-NRCS (http://plants.usda.gov)
Figures 24, 35	Biocontrol of Weeds in the West
Figures 25, 60, 62, 65	K. Loeffelman, University of Idaho
Figures 29, 41, 53	J. Johnson, University of Idaho
Figures 30, 31	R. Gillespie, University of Idaho
Figures 33, 37, 38, 45, 50-52	USDA APHIS
Figures 44, 54	www.nysaes.cornell.edu:80/ent/biocontrol/weedfeeders
Figure 63	BioQuip Catalog
Figure 70	S. Kegley, USDA Forest Service